# ANNUAL REPORT
# OF
# PROGRAM ACTIVITIES

# NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

## PART 1

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

# ANNUAL REPORT

# OF

# PROGRAM ACTIVITIES

# NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

## Fiscal Year 1973

## Part I

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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# NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

## ANNUAL REPORT

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# ANNUAL REPORT
July 1, 1972 through June 30, 1973
National Institute of Neurological Diseases and Stroke
National Institutes of Health

## The Acting Director's Report

During FY 1973 the NINDS has been beset by more than the usual array of administrative, fiscal and programmatic problems. The resignation of the incumbent Director, Dr. Edward F. MacNichol, Jr., became effective in January 1973, and he was succeeded in turn by two Acting Directors, Drs. Robert Q. Marston (from January to April 1973) and Donald B. Tower (from May 1973 to the present). The process of selection of a new permanent NINDS Director has been complicated by the resignation in December 1972 of the Director, NIH, and the subsequent 5 month interval before the naming of his successor. In addition there was a turnover of the NINDS Associate Director for C&FR, with Dr. Leo Fox replacing Dr. Warren Huber, and a replacement for the NINDS Director for IR (who asked to step down one year ago) remains deferred pending appointment of the new NINDS Director. These uncertainties and shifts in three of the four top administrative posts within NINDS have been less than salutary for institute programs, especially during a year of severe budget reductions and personnel restrictions. In the course of Congressional and White House actions on vetoed FY 1973 appropriations, the NINDS "lost" approximately $10 million between the original President's FY 1973 budget and the final continuing resolution level of operations. Like other institutes, the NINDS was also faced with further ceiling-imposed reductions in personnel and a virtual freeze on promotions because of grade-point average restrictions. The cumulative effects have created staff morale problems and serious curtailments of programs at a time when pressing national needs and significant scientific break-throughs confront the NINDS and demand action.

In its extramural programs (EP), the NINDS has seen a 40 percent reduction in the number of active research grants over the period FY 1968 to FY 1973 as a consequence of the decreasing availability of funds and the increasing average cost of each grant. Simultaneously the number of applications for NINDS research grant support has continued to increase, so that the current year's total now exceeds that for the year prior to removal of vision research from NINDS program responsibility. In the face of a fairly constant percentage (50 percent) of applications recommended for approval and in the face of increasing numbers of applications and of decreasing funds, less than 30 percent of the total competing grant applications recommended for approval can now be funded, and the percentage for the new applications is even lower (less than 10 percent). Moreover, the President's FY 1974 budget reflects the decision to phase-out training programs, a process initiated in January 1973. The impact of the loss of the NINDS training programs on future manpower planning is currently under evaluation by contracts examining the present status of and future needs for manpower in the fields of neurological and communicative disorders.

Despite the stringent budgetary limitations on NINDS research grant programs, some needed support in selected targeted areas has moved ahead with the activation of 3 out of 6 recommended acute spinal cord research centers and the funding of 10 stroke acute care research units throughout the country. Focus on several other areas is indicated by the current review in progress by an NANDS Council subcommittee on research on the brains in aggressive behavior, by the currently active National Commission on Multiple Sclerosis, and by the proposal for a task force (probably under the aegis of the NANDS Council) on central nerve regeneration. Support has continued for 13 stroke clinical research centers, 5 epilepsy and 5 head injury clinical research centers, 3 multiple sclerosis and 3 parkinsonism clinical research centers, 5 communicable disorders outpatient or clinical research units, 2 clinical research centers for neuromuscular disorders, and 44 program projects and targeted research programs in speech and hearing, demyelinating disorders, nerve injury and regeneration parkinsonism, spinal cord injuries, sensory and perceptual disorders, and general neurological disorders. Among individual research grants funded by NINDS a number of significant and intriguing studies are in progress: the role of nerve growth factor in spinal cord (central) regeneration; synaptic plasticity; isolation of receptor proteins; seizures and retardation of brain growth and deletion of neurons; cerebellar locus for dilantin action; the valium group of drugs as anticonvulsants; correlation of the recurrence rate of seizures after suspension of anticonvulsant therapy and the type of seizures (highest recurrence for multiple seizure types); immunological approaches to possible antiglial factors in MS and antineuronal factors in ALS; ischemic lesions in stroke and in cord and head injuries; EEG changes often the earliest sign of slow virus infection; comparative studies of virally-induced demyelination (PML, canine distemper, mouse hepatitis, visna in sheep); reproduction in animals of myasthenia gravis after injection of antibodies to muscle receptor protein and alleviation with neostigmine; evaluation of damage by constant noise to the cochlea; vestibular function in Meniere's desease; diagnostic applications of electroacoustic impedance; role of trace metals (Zn, etc.) in mechanisms of olfaction and taste.

The NINDS collaborative and field research (C&FR) programs complement the research grant programs with a combination of in-house and contract studies directed at solving specific problems in the neurological and communicative disorders. At present the research grants account for about 60 percent of the NINDS annual budget, whereas C&FR contracts represent about 8 percent of budget. A major program has been the collaborative perinatal project which is now in its final stages of data analysis and interpretation in anticipation of termination in FY 1976, with the completion of monographs in the 10 major areas of concern. After reorganization during FY 1973 the C&FR in-house and contract programs now include the following other areas: epilepsy, with clinical studies on newly developed anticonvulsant drugs and correlations of drug blood levels and conversion to metabolites with therapeutic responses; head injury and stroke, including a cooperative study on transient ischemic attacks; communicative disorders, with developments of diagnostic tests for hearing impairment in early childhood, of sensory cues to improve the lipreading ability of deaf adults, and of "hi-fi" hearing aids; epidemiology, with a major focus on the NINDS-ALS facility on Guam, as well as several studies on stroke epidemiology; infectious diseases, with major emphasis on viral infections of the measles, encephalitis and cytomegalovirus types; and biomedical engineering and biometry capabilities.

Intramural research (IR) by NINDS is conducted by some 150 scientists on the NIH campus. During FY 1973 the new Developmental and Metabolic Neurology Branch was created to concentrate on biochemical and clinical studies of genetically-determined metabolic disorders of the nervous system, and the laboratory of Central Nervous System Studies was transferred from C&FR to IR for continuation of research on slow virus infections of animals and man. Other realignments within existing laboratories and branches are increasing focus on the surgical approaches to epilepsy, stroke, tumors, trauma, movement disorders, on biochemical and tissue culture approaches to central nerve regeneration, and on experimental studies of cerebrovascular pathology. A rich yield of stimulating and important findings has emerged from IR projects during FY 1973: solubilization and characterization of the Na-K-ATPase enzyme that promise elucidation of mechanisms responsible for transducing cellular energy into transport of ions across neural membranes; role of astrocytes in the fluid and ionic balance in brain tissues; dynamics of brain edema after ischemic insults; techniques for observing myelination in vivo; roles of immune response and genetic histocompatibility in axonal regeneration; transmembrane transport as an indicator of cell differentiation--evaluation with short-chain lipophilic acid inhibitors; characteristics of isolation sodium-specific ionophores in artificial bilayer membranes; development of a variety of special electrode devices for neural control prostheses; elucidation of sensory (visual) transduction mechanisms and of the rhythmicity of pacemaker neurons; ontogenetic shifts in seizure propagation from subcortical to cortical loci; isolation of apparently specific glycoproteins from gliomas; evaluation of spinal cord circulation by radioisotope angiography; arterial ischemic mechanisms underlying Duchenne's muscular dystrophy; prednisone therapy for myasthenia and myotonia; fluorimetric monitoring in situ of NADH changes in cerebral cortex during seizures; isolation and therapeutic trials of the respective catabolic enzymes genetically deleted from tissues in Fabry's, Gaucher's and Tay-Sachs diseases; demonstration of the slow viral transmission of the heredo-familial form of Creutzfeld-Jakob disease; suggestive evidence for a slow virus cause for Alzheimer's disease and for some forms of multiple sclerosis; and partial purification and successful concentration of atypical slow virus agents (kuru, scrapie, etc.) preliminary to detailed characterization.

Such studies involved during FY 1973 a total of 505 patient admissions (11,686 patient days), with 65 major and 326 minor (mostly biopsy) operations, and 144 active research projects. Among the most promising research developments in the IR program are the work on genetically-determined metabolic disorders and the slow virus studies. In the latter context the papova viruses (especially the JC strain) isolated from demyelinating lesions of human cases of progressive multifocal leukoencephalopathy (PML), when injected into brains of guinea pigs and other species, have proved to be the most potent tumorigenic agents yet found, with a high percentage of medulloblastomas induced. The implications of such findings are wide-ranging indeed.

A major concern of the NINDS continues to be the Institute equal employment opportunity (EEO) programs. Despite the curtailments imposed during FY 1973 by employment ceilings and grade-point average controls, yeoman service has been rendered by the Institute EEO coordinator and its EEO Committee. Two NINDS employees received EEO special achievement awards and one supervisor received honorable mention for the DHEW award on behalf of the federal women's program. During FY 1973 there were 21 NINDS employees in Upward Mobility College and 16 employees have applied for the Stride program. Programs to increase employee skills and to develop supervisory training continue, and the affirmative action program implementation plans were completed and forwarded to the NIH-EEO officer. Science students from Tougaloo College in Mississippi toured NINDS laboratories to observe applications of neurosciences firsthand. And the "frontiers in research and training in neurosciences" for minority postdoctoral scientists is being continued for the second year at the Marine Biological Laboratory, Woods Hole, Mass. This last program has proved most successful. It would be a major tragedy if the NIH-wide phase-out of training programs prevents the continuation of this most rewarding activity. NINDS will support and participate in a conference on exposition to the minority community of various NIH biomedical research programs. At present the major shortcoming in the NINDS-EEO program is the lack of minority and women employees in key administrative, scientific and training positions, but efforts continue to rectify these imbalances.

In the present climate of concerns with cancer and heart research and with applied directed research, special emphasis on neurological and communicative disorders needs reiteration. The NINDS is concerned with problems that afflict some 40 million Americans at an annual cost to the nation conservatively estimated at over 20 billion dollars. Diseases of the nervous system are among the most chronic, debilitating and frightening diseases that afflict mankind. Few of these diseases have yielded to the current intensive research programs, but the challenges of understanding the human nervous system and its disorders are attracting more and more of the best minds in science and medicine. Research in the neurosciences is today the most exciting of frontiers and over the next decade can provide us with some of the most significant break-throughs of this century. Our ability to exploit these opportunities and the efficacy with which we can overcome neural and communicative disorders must not be vitiated by expediencies of the moment. At the present time the NINDS is apportioned annually only $2.50 per patient to invest in research on neurological and communicative diseases that are annually costing our nation $500 per patient. The familiar example of the cost of caring for victims of poliomyelitis before the development of vaccines in comparison to the cost of immunizing the nation against polio clearly illustrates the alternatives. Surely the alternative of false economies is an alternative that the nation cannot afford.

## The Office of Public Affairs

The Office of Public Affairs of the NINDS is organized to provide public relations and information assistance to the Institute staff and to make to the scientific community and the public the latest information on research advances on neurological and sensory disorders. Information activities are designed to achieve greater understanding and appreciation of NINDS programs as well as research accomplishments on the part of Congress, biomedical institutions, professional societies, the medical profession, voluntary health agencies, and the public.

During the past year, the Information Office was reorganized and given a new title, the Office of Public Affairs. The office was divided into four sections which reflect more accurately the various functions of the office. The sections are Public Information and Press, Publications and Reports, Community Relations, and TV, Radio, and Films.

## Public Information and Press

The major function of the press section is to make available research findings on neurological and sensory disorders. Reporters or other media staff are called or call a number of times each week for assistance in arranging interviews with NINDS scientists, obtaining the names of experts on special research subjects among Institute grantees, and information from Institute files. Members of the Public Affairs staff usually join reporters in interviews with Institute staff on subjects of particular interest to be of assistance and additionally to obtain material for NIH Record stories, for special reports, leads on feature stories, and to obtain material for use in answering public inquiries.

During the year, the press section prepared a number of press releases and announcements. It also assisted NIH and the Department in the preparation of a number of releases including the establishment of the National Multiple Sclerosis Commission. Assistance was also given in setting up and managing the press conference for the Commission to announce the setting up of a large research prize.

The Press and TV sections assisted with advance publicity for the Second Annual Meeting of the Society for Neurosciences in Houston and in managing the press at the meeting. Seven stories from the meeting appeared in the Houston Post, Houston Chronicle and over the AP and UPI wire services.

During the year, 48 stories were written for the agency house organ, the NIH Record. Also some 20 articles were submitted for the NIH Research for Health series and sent to 60 small daily and weekly newspapers with a readership of 11 million.

The press section has greatly increased its participation this year in the NIH News and Features Service. The Service uses illustrated articles concerning work conducted or supported by NIH and is distributed to a mailing list of science writers, newspapers, and more than 100 professional journals, nationally and internationally with a potential readership of seven million. Many of these stories were written by information staff; others were written by Features Service staff with editorial and clearance assistance from the press section.

Information staff members prepared a number of messages for the signature of Department officials or the President.

## Publications and Reports Section

### Publications

The Publications and Reports Section processed, published, and distributed many publications, both for the general public and for various scientific audiences. In the past several years, the services relating to production of scientific and professional publications have been substantially increased. These services are usually requested by various administrative units of the Institute, and usually include responsibility for all stages of production.

The Monograph Series contains neurological science contributions of Institute staff, committees, and consultants. Included are reviews, reports, nomenclature, classification and methodology aids, and proceedings of meetings sponsored by the Institute. The thirteenth in this series, "The Epidemiology of Epilepsy: A Workshop," is scheduled for delivery in July 1973. This publication contains some 28 papers on all aspects of the subject, and will be distributed to 10,000 physicians, scientists, libraries, and other individuals and organizations with an interest in this field.

An NINDS Bibliography Series was started in Fiscal Year 1973 with publication of bibliographies on Brain Death and Blood Level Determination of Antiepileptic Drugs. These are computer-assisted compilations of the Institute's Applied Neurologic Research Branch, with production and distribution services supplied by the section.

The latest of the Profile Series, NINDS Research Profiles 1972, was published on research advances in seven major disease areas. This is a 70-page booklet containing special Congressional reports and an opening statement by the Director on general program directions.

Research Grant Support and NINDS Training Grant and Awards Programs, two booklets serving as guides to prospective applicants for grants, were updated, revised and reprinted in Fiscal Year 1973.

The Institute's highly popular Hope through Research series was expanded, revised, and reprinted as necessary. These health information leaflets, now numbering 18, with three in both English and Spanish, are by far the most widely circulated of the Institute's publications.

To help meet the need to attract young people towards careers in neuroscience, the Institute published "Discovering Yourself in the Brain Age," a colorful new booklet on the brain, the history of brain and nervous system research. This booklet is being widely circulated to high schools and colleges, and is expected to be a "best seller."

Other items also produced by the Publications Section included a directory of NANDS Council members; several issues of an EEO newsletter; a special list of epilepsy publications; a job vacancy form for the NINDS Personnel Office; an NINDS Personnel Merit Promotion pamphlet; a directory of former management interns, and a listing of voluntary agencies working in the neurological and sensory disease fields.

The Public Affairs Office prepared seven special congressional reports and an opening statement for presentation by the Director at the annual Appropriations Hearings. The office also prepared a document entitled "Highlights of Research," presenting interesting and important research events for use in Congressional testimony. Several supplemental documents were also prepared in connection with the hearings, including an extensive review of slow virus research.

Intramural scientists conducted a complete review of in-house research which was assembled in a large document entitled: Biomedical Research: A Time for Accountability. The materials for this publication were edited or rewritten extensively by the Public Affairs Office.

The Office of Public Affairs is now distributing 49 publications on neurological and sensory disease problems. Of these, 29 were written by the staff for the general public, and 20 others covering scientific subjects were prepared for distribution to various professional groups. Altogether, 382,672 of these publications were distributed during the past year.

During the year, 58 journals were reviewed on a weekly and monthly basis, with research weeklies and program items prepared for the Director, NIH, and the Assistant Secretary for Health and Scientific Affairs. In addition, material obtained from the journals was used by Public Affairs staff to respond to public inquiries and in the preparation of special Congressional reports. Arrangements were made for Institute lectures, including preparation of announcements, programs, and flyers.

## Community Relations Section

The new Community Relations Section coordinates information activities which affect NINDS relations with grantee institutions, voluntary health agencies, professional societies, the NANDS Council, and Institute employees. This includes the scheduling and manning of program exhibits which the Institute sends to medical meetings. This section is also now responsible for the Office's efforts in responding to public inquiries.

As usual, staff members spent a great deal of time responding to letters and inquiries by phone. More than 2,500 inquiries required individually prepared letters in answer to specific questions. Congressional mail was very heavy this year as a result of budget problems and in response to constituents urging the establishment of the MS Commission. Additional thousands of inquiries were answered through the use of printed materials. An estimated 100,000 requests of all types were received this past year.

This section, with assistance from a staff member in the press area, provides assistance to the Equal Employment Opportunity Program. In addition to representing the Information Office and participating in the work of this special program, the section staff prepares and distributes an EEO newsletter. Staff also assists with award ceremonies, provides photographic services, and writes special feature stories on occasion.

The Community Relations staff performs many services for the 20 national voluntary agencies and 20 professional societies in the neurological and sensory area. These agencies are members of an overall committee called the National Committee for Research in Neurological Disorders. Various types of assistance is provided this committee by The Office of Public Affairs. Staff members meet with these agencies in developing programs and coordinating educational and publicity campaigns. Much of the Institute literature published for the general public is distributed by the voluntary agencies.

In an effort to further communication with the national voluntary agencies as well as with the NINDS Advisory Council and professional societies, the Information Office publishes the NINDS Review, a bi-monthly newsletter. Items in this publication are obtained directly from officials of these various organizations and are also excerpted from house organs, press releases, periodicals and professional journals in the bio-medical field.

In accordance with Public Law 92-463, the Federal Advisory Committee Act, the NINDS Information Office distributes on request summary minutes of activities of the Institute's public advisory groups and the names of members of each group to interested individuals. This year there have been 33 requests for this information.

Although the Public Affairs Office has reduced its exhibit activity (in response to a Department request), exhibits were shown during the year at the AAOO in Las Vegas, the AAN in Boston, the ASHA in San Francisco, and at the ANA in Montreal. The special publications exhibit has now been displayed 43 times in schools, libraries, and in hospital lobbies. Several hundred publications have been distributed or requested as a result of each week-long showing.

## TV, Radio, and Films

A number of NINDS scientists participated in television and radio interviews concerning their research. This included a special telephone hookup in which the Institute Director participated on a radio talk show over KMOX radio, St. Louis, Missouri, on the subject of detecting cerebral death. In another instance, the WRC-TV producer of a program called "Perspective" came to NINDS to interview Drs. Brady, Penry, and Sever on "rare" diseases and obtained background information for one or more planned programs about neurological research.

This section brought together the diverse artistic, photographic and editorial work necessary to publish the 32-page booklet on brain research, "Discovering Yourself in the Brain Age." Novel ways for NINDS to present it for sale were worked out with the Government Printing Office which will fill the orders from their stock. Also, TV and radio spots are ready to go to stations throughout the country but are being held for simultaneous release with the GPO's advertising materials.

Eighteen 50-minute video tapes were produced in a series of lectures in an "Intensive Course in Neuromuscular Diseases," held in Houston in November. The tapes are being offered for sale to medical schools, centers, and hospitals for their education programs.

Film sequences were shot in the NINDS epilepsy research laboratories and prepared for inclusion in an HEW TV series, "You."

Five scientists participating in the Society of Neurosciences' Second Annual Meeting appeared on three Houston TV news and three radio programs. Arrangements for their radio and TV press operations were directed by members of this section.

The Office's photo file is being constantly enlarged and updated. The aim is to fill more effectively requests for photos from 30-to-40 magazine and book publishers each year, and from other sections (Publications and Press) of the Public Affairs Office for use in news releases, feature articles and Institute and NIH/OD publications. A recent project has been the photographing of three stroke research center projects for a 25-page portfolio being produced by the NIH/OD's Press Branch.

# Table of Organization

## Intramural Research

## National Institute of Neurological Diseases and Stroke
(Personnel on hand May 1973)

### Office of the Director

Director of Intramural Research - Henry G. Wagner, M.D.
Assistant Director of Intramural Research - Richard L. Irwin, Ph.D.
Act. Clinical Director - John M. Van Buren, M.D., M.Sc.
Administrative Officer - Glenn E. Hammond
Mathematician - Rosalind B. Marimont
Research Biologist - Halsey M. Marsden, Ph.D.
Administrative Officer - J. Loring Jenkins
Photographer (Scient. & tech.) - Wesley Pearson, Jr.
Photographer (Medical) - Jerry M. Ellis
Administrative Assistant - John H. Jones
Biological Laboratory Technician (Animal) - George R. Duvall
Biological Laboratory Technician (Animal) - Adrian P. Loftis
Secretary (Steno) - Margret L. Shipley
Travel Assistant - Ida M. Chernikoff
Administrative Technician - Doris R. Perry
Procurement Clerk - Patricia D. Williams
Budget Clerk (Typing) - Heather A. Roberts
Clerk-Typist - Judy R. Harvey
Laboratory Worker - John R. Bowers
Laboratory Worker - Kenneth M. Oglesbee
Laboratory Worker - Stevan Vanderhorst

### Section on Technical Development

Computer Programmer - William H. Sheriff, Jr.
Engineering Technician - Benjamin H. Shellman
Scient (R)(T) - Raymond M. Fish, Ph.D.

### Medical Neurology Branch

### Office of the Chief

Chief, Medical Neurology Branch - W. King Engel, M.D.
Biological Laboratory Technician (Microbiol.) - Guy G. Cunningham
Biologist - Joseph G. Brown
Physiologist - Carolyn Derrer
Biologist - Mary A. Oberc
Surgeon - Mark S. Horwich, M.D.
Surgeon - Michael L. Swerdlow, M.D.
Surgeon - Barry E. Levin, M.D.

Surgeon - Richard W. Price, M.D.
Surgeon - Alan D. Prince, M.D.
Surgeon - Bernard M. Patten, M.D.
Surgeon - Peter K. Re, M.D.
Surgeon - Barry W. Festoff, M.D.
Secretary - Lucy L. Riley
Microbiologist - Priscilla B. Chauvin
Secretary (Dict. Mach. Trans.) - Gertrude E. Wright
Clerk (Dict. Mach. Trans.) - Anne K. Bowen
Biological Laboratory Technician - Pauline R. Middleton
Clerk (Dict. Mach. Trans.) - Ann M. Macker
Histopathology Technician - Thelma R. Fletcher
Biological Aid (Micro) - Joseph S. Sciabbarrasi
Biological Aid (Animal) - Gregory Hubbard
Clerk-Typist - Beverly Williams
Laboratory Worker - Matthew P. Meadows
Visiting Fellow - Husseiny I. Dessouky
Visiting Fellow - Neelupalli B. Reddy

## Section on Clinical Applied Pharmacology

Super. Research Pharm. (Gen.) - Richard L. Irwin, Ph.D.
Research Physiologist - Jay B. Wells , Ph.D.
Biologist - Katharine L. Oliver
Secretary (Dict. Mach. Trans.) - Emma P. Dick

## Surgical Neurology Branch

### Office of the Chief

Chief, Surgical Neurology Branch - John M. Van Buren, M.D., M.Sc.
Medical Officer (Neuro. Surg.) - Choh-luh Li, M.D., Ph.D.
Research Psychologist (Clin.) - Paul Fedio, Jr., Ph.D.
Biologist - Rosemary C. Borke
Psychologist - Laura S. Rosenthal
Surgeon - Adelbert L. Albright, M.D.
Surgeon - Maxim O. Koslow, M.D.
Surgeon - Daniel B. Ahlberg, M.D.
Surgeon - John C. Madigan, M.D.
Surgeon - Hugh H. West, M.D.
Visiting Fellow - Naomi Mutsuga, M.D.
Medical Technician - Vivian Betton
Clerk (Dict. Mach. Trans.) - Helen M. Andregg
Clerk-Stenographer - Myrtle M. Sullivan
Secretary (Typing) - Lillian L. Wease
Clinical Psychologist - Christiane S. Cox
Biological Laboratory Technician (Micro) - Charles E. Sartor
Secretary (Steno.) - Olga Williams

## Section on Applied Research in Surgical Neurology

Medical Officer (Neuro. Surg.) - Ayub Khan Ommaya, F.R.C.S.
Visiting Scientist - Susumu Nakatani, M.D.
Biologist - Kenneth Rich
Biological Laboratory Technician (Animal) - Calvin Hawkins
Biological Laboratory Technician (Animal) - Mitchell Major
Biological Laboratory Technician (Animal) - M. Arthur Banks
Biological Laboratory Technician (Animal) - Clifford A. Seay
Clerk (Dict. Mach. Trans.) - Lois A. Brown

## Section on Neuroradiology

Research Medical Officer - Giovanni Di Chiro, M.D.
Clerk-Stenographer - Linda Crouse
Research Physiologist - Eugene L. Timins, M.D.

## Electroencephalography Branch

### Office of the Chief

Chief, EEG Branch - Cosimo Ajmone Marsan, M.D.
Surgeon - Darrell V. Lewis, Jr., M.D.
EEG Technician - Rosemarie Schilling
EEG Technician - Joan Trettau
Secretary (Dict. Mach. Trans.) - Mattie L. Jackson
Clerk-Typist - Fonda S. Hampton

### Section on Clinical Neurophysiology

Surgeon - Robert A. Levine, M.D.
Surgeon - John S. Ebersole, M.D.
Biologist - Minnie Toure

## Developmental and Metabolic Neurology Branch

### Office of the Chief

Chief, DMNB - Roscoe O. Brady, M.D.
Secretary (Steno.) - Barbara W. Critzer
Secretary (Steno.) - Sandra S. Costanzo
Biological Laboratory Technician (Animal) - Alexander Wheaton
Biological Aid - Fred Lipschultz

### Section on Enzymology and Genetics

Senior Staff Fellow - Richard H. Quarles, Ph.D.
Staff Fellow - Peter G. Pentchev, Ph.D.
Senior Staff Fellow - Peter H. Fishman, Ph.D.
Staff Fellow - John F. Tallman, Ph.D.

Chemist - Roy M. Bradley
Chemist - Jane M. Quirk
Biological Laboratory Technician - George E. Mook

### Section on Neurochemical Methodology

Research Chemist - Andrew E. Gal, Ph.D.
Biological Laboratory Technician (Biochem) - Frank J. Fash

### Section on Neurological Development

Associate Chief, DMNB - Anatole Dekaban, M.D., Ph.D.
Research Chemist - Dennis F. Cain, Ph.D.
Surgeon - Andrew W. Zimmerman, M.D.
Surgeon - Marvin Zelkowitz, M.D.
Research Chemist - George Constantopoulos, Ph.D.
Medical Technician - Jan K. Steusing
Visiting Fellow - Ken Fujitani, M.D.
Biological Aid - D. Douglas Fratz

## Laboratory of Neuropathology and Neuroanatomical Sciences

### Office of the Chief

Chief, LNNS - Igor Klatzo, M.D., M.Sc.
Biological Laboratory Technician (Animal) - Willie Perkins
Biological Laboratory Technician (Animal) - Albert V. Cantu
Biological Laboratory Technician (Animal) - Melvin H. Carroll
Secretary (Dict. Mach. Trans.) - Virginia A. Masterson
Clerk-Stenographer - Patricia A. Bustin

### Section on Neurocytology

Research Physiologist - Milton W. Brightman, Ph.D.
Surgeon - Jonathan L. Costa, M.D.
Biological Laboratory Technician - Clara E. Mowry
Visiting Fellow - Erik Westergaard, M.D., Ph.D.

### Section on Functional Neuroanatomy

Medical Officer (Res.) - Thomas S. Reese, M.D.
Biological Laboratory Technician (Gen.) - Frank D. Nolan
Biologist - Avery D. Nelson, Ph.D.

### Section on Cerebrovascular Pathology

Biological Laboratory Technician - Joseph T. Walker, Jr.
Surgeon - Frank G. Berson, M.D.

## Section on Experimental Neuropathology

Medical Officer (Path.) - Jan Cammermeyer, M.D.
Biologist - Iris Mercado
Editorial Assistant - Jane T. Phelps
Biological Aid - Sophia Grabinski

## Section on Cellular Neuropathology

Medical Officer (Research) - Henry deF. Webster, M.D.
Biological Laboratory Technician - Maureen F. O'Connell
Biologist - John R. Martin

## Section on Cellular Neurochemistry

Research Biologist - Janet V. Passonneau, Ph.D.
Biologist - Vivian R. Lauderdale
Research Chemist - Joan P. Schwartz, Ph.D.
Staff Fellow - Wesley D. Lust, Ph.D.
Biologist - Helen J. Osborne

## Section on Neurocytobiology

Medical Officer (Res.) - Maria Spatz, M.D.
Biological Laboratory Technician (Micro.) - Madora E. Swink
Biological Laboratory Technician (Micro.) - Jo Ann Holmes

## Laboratory of Biophysics

Chief, Laboratory of Biophysics - William J. Adelman, Jr., Ph.D.
Super. Research Physiologist - Robert E. Taylor, Ph.D.
Research Biophysicist - Kenneth S. Cole, Ph.D.
Research Physicist (Gen.) - Richard Fitzhugh, Ph.D.
Research Physiologist (Neurophys.) - Daniel L. Gilbert, Ph.D.
Electrical Engineer (Instru.) - Leonard Binstock
Research Physicist (General) - Gerald M. Ehrenstein, Ph.D.
Mathematician - John Shaw
Research Physicist (Gen.) - Harold Lecar, Ph.D.
Staff Fellow - Frederick Sachs, Ph.D.
Electronic Technician - Herbert A. Walters
Biologist - Allen D. Spencer, Jr.
Secretary (Stenography) - Vernita Bergmeyer
Clerk-Steno - Sandra J. Cotter
Biological Aid - Mark A. Holbert

## Laboratory of Neurochemistry

### Office of the Chief

Chief, Laboratory of Neurochemistry - Donald E. Tower, M.D., Ph.D.
Secretary (Stenography) - Shirley A. Burdette

Clerk-Steno - Pamela A. Barnicoat
Clerk-Steno - Michele A. Cahill

## Section on Amino Acid Chemistry

Chemist - Oscar M. Young
Surgeon - Thomas H. Gill, M.D.
Biological Aid (Gen.) - Kenneth Palm

## Section on Physiology and Metabolism

Research Chemist - Eberhard G. Trams, Ph.D.
Chemist - Carl J. Lauter

## Section on Enzyme Chemistry

Research Chemist - R. Wayne Albers, Ph.D.
Physiologist - Stephen S. Goldman, Ph.D.
Chemist - George J. Koval
Physical Science Technician (Chem.) - Eunice L. Summers
Visiting Fellow - Dou H. Jean, Ph.D.

## Section on Neural Development and Regeneration

Medical Director - Lloyd Guth, M.D.
Physiologist - Herbert Yellin, Ph.D.
Biological Laboratory Technician (Micro.) - Janina D. Ziemnowicz
Surgeon - Andrew A. Zalewski, M.D.
SR HSO - George F. Creswell

## Laboratory of Molecular Biology

## Office of the Chief

Chief, Laboratory of Molecular Biology - Ernst Freese, Ph.D.
Secretary (Stenography) - Mary W. Taylor
Biological Aid - Tanya R. Brown
Clerk-Typist - Jacqueline L. Law
Biological Aid - Walfredo Leon
Clerk-Stenographer - Sylvia Z. Levinson
Staff Fellow - Dorothy A. Stroup, Ph.D.
Microbiologist - Peter Cooney, Ph.D.

## Section on Developmental Biology

Research Biologist - Elisabeth G. M. Freese, Ph.D.
Surgeon - Jeffrey L. Simmons, M.D.
Senior Staff Fellow - Michael L. Hampton, Ph.D.
Senior Staff Fellow - Martin D. Diesterhaft, Ph.D.
Staff Fellow - Richard C. Henneberry, Ph.D.
Chemist - Enid M. Galliers

Chemist - Cheryl L. Marks
Visiting Fellow - Yong K. Oh, Ph.D.
Visiting Fellow - Chandan Prasad, Ph.D.
Visiting Fellow - Tomio Ichikawa, Ph.D.

## Section on Regulation of Nucleic Acid Syntheses

Research Chemist - Robert A. Lazzarini, Ph.D.
Chemist - Leslye E. Johnson
Staff Fellow - Michael F. Murphy, Ph.D.

## Laboratory of Experimental Neurology

### Office of the Chief

Chief, Laboratory of Experimental Neurology - William F. Caveness, M.D.
Visiting Associate - Hideo Ueno, M.D.
Visiting Associate - Akira Tanaka, M.D.
Biological Laboratory Technician (Animal) - Kenneth Hess
Clerk-Typist - Marguerite H. Smiley
Medical Technician - Sandra K. Crites

## Laboratory of Neurophysiology

### Office of the Chief

Chief, Laboratory of Neurophysiology - Michelangelo G.F. Fuortes, M.D.
Electronics Technician - Anthony F. Bak
Biological Laboratory Technician (Animal) - William L. Beane
Secretary (Steno.) - Johanna L. Barnett
Clerk-Stenographer - Susan A. Jacobsen
Histologist - Leona M. Stewart

### Section on General Physiology

Research Neurologist - Ludwig von Sallmann, M.D.
Chemist - Patricia A. Grimes
Senior Staff Fellow - Daniel L. Alkon, M.D.
Surgeon - Elliott J. Simon, M.D.

### Section on Sensory Physiology

Medical Officer (Research) - Thomas G. Smith, Jr., M.D.

### Section on Cell Biology

Research Biologist - Arnaldo Lasansky, M.D.
Visiting Scientist - P. Lorenzo Marchiafava, M.D.
Biological Laboratory Technician (Micro.) - Julia M. Lohr

## Section on Neuronal Interactions

Head, Section on Neuronal Interactions - Henry G. Wagner, M.D.
Senior Research Biophysicist - Edward F. MacNichol, Ph.D.
Senior Surgeon - John McReynolds, M.D.
Staff Fellow - Ferenc I. Harosi, Ph.D.
Staff Fellow - Peter D. Detwiler, Ph.D.

## Laboratory of Neural Control

### Office of the Chief

Chief, Laboratory of Neural Control - Karl Frank, Ph.D.
Medical Officer (Res.) - Robert E. Burke, M.D.
Super. Research Psychologist - Herbert C. Lansdell, Ph.D.
Biological Engineer - Edward M. Schmidt, Ph.D.
Senior Surgeon - Frederick T. Hambrecht, M.D., Ph.D.
Surgeon - David L. Guyton, M.D.
Surgeon - John V. Walsh, Jr., M.D.
Secretary (Stenography) - Dorothy M. Wilbur
Psychologist - Kathleen K. Davis
Microelectronics Technician - George F. Laird, III
Electronics Technician - Martin J. Bak
Clerk (Dict. Mach. Trans.) - Gloria K. Hall
Engineering Technician - George M. Dold
Physiologist - Joan S. McIntosh

## Laboratory of Perinatal Physiology

### Office of the Chief

Chief, Laboratory of Perinatal Physiology - Ronald Myers, M.D., Ph.D.
Research Chemist - Americo Rivera, Jr., Ph.D.
Research Psychologist - Shun-ichi Yamaguchi, Ph.D.
Chemist - Jose Martinez de Jesus
Biological Laboratory Technician (Animal) - Esteban Monell-Torrens
Biological Laboratory Technician (Animal) - William Rodriguez
SA Surgeon - Francis W. Gamache, M.D.
Surgeon - Gary S. Kopf, M.D.
Surgeon - David M. Mirvis, M.D.
Chemist (Biochemistry) - Carmen L. Freixas
Secretary (Steno) - Elsie M. Walter
Biological Laboratory Technician (Biochem.) - James H. Cane
Biological Laboratory Technician (Animal) - Joseph C. Pazdersky
Biological Laboratory Technician (Animal) - Bernardino Santana

## Laboratory of Central Nervous System Studies

### Office of the Chief

Chief, LCNSS - D. Carleton Gajdusek, M.D.

Super. Research Microbiologist - Clarence J. Gibbs, Ph.D.
Medical Officer (Research) - David M. Asher, M.D.
Senior Surgeon - Paul Brown, M.D.
Surgeon - Richard A. Ferber, M.D.
Surgeon - Stephen L. Wiesenfeld, M.D.
Veterinarian - Herbert L. Amyx, D.V.M.
Microbiologist - Nancy Rogers
Soc. Sci. Analyst - E. Richard Sorenson
Soc. Sci. Analyst - Judith B. Meyer
Anthropologist - Ralph M. Garruto
Biological Laboratory Technician - Michael P. Sulima
Microbiologist - Nancy P. Luber
Biological Laboratory Technician - Alfred E. Bacote
Biological Laboratory Technician (Micro) - Helena L. Gilbert
Secretary (Steno) - Marion F. Poms
Clerk (Typing) - Linda Z. Poole
Translator - Josede Figirliyong
Biologist - Monica A. Lewis
Student Trainee - Steven G. Ono
Health Technician - Ivan M'Bagintao
Animal Caretaker - Wesley B. Shirk
Clerk-Typist - Lois E. Ludeman
Animal Caretaker - Edwin E. Horst
Staff Fellow - John J. Hooks, Ph.D.
Secretary (Dict. Mach. Trans.) - Juliette L. Harvey
Animal Caretaker - Albert Bontrager
Biological Aid (Gen.) - Douglas G. Black
Biological Aid (Gen.) - Timothy Slater
Biological Aid (Gen.) - Elizabeth M. Adler
Biological Aid (Gen.) - Nancy J. Henly
Animal Caretaker - Thomas R. Bender
Animal Caretaker - James H. Webster
Biological Aid (Gen.) - Charles E. Thomas Jr.
Biological Aid (Gen.) - Kathleen A. Maynor

# Annual Report of the Scientific Director of the National Institute of Neurological Diseases and Stroke

July 1, 1972 through June 30, 1973

The Intramural Research Program is that part of the National Institute of Neurological Diseases and Stroke authorized to conduct research related to the nervous system in its own laboratories and clinics. The creative research efforts of the staff utilize both basic and applied approaches of a wide variety to investigate nearly every aspect of normal and deranged nervous systems.

One-hundred and fifty-one scientists, 123 of them at the doctorate level, comprising 24 scientific disciplines, pursue one-hundred and fifty research projects with vigor and enthusiasm. As judged by scientific productivity and the laudatory recognition given by the national and international research communities, the Intramural Research Programs have been outstandingly successful during the past year. One-hundred and sixty-five major research communications have been published in the major science journals. Many more abstracts and speeches have been presented before both domestic and foreign learned societies and research departments in universities or other research institutions. The Intramural Research staff eagerly looks forward toward continued and increased support of programs which will enable them to help solve the problems created by the neurological diseases. The expertise of the staff is outstanding, their accomplishments are noteworthy, and their hopes are high that their efforts will ameliorate and eventually eliminate the disability and destruction caused by these dreadful diseases.

The program planning activities of the Office of the Director of Intramural Research have in part been revised and expanded during the past year:

1. A new fiscal system CLEAR (Computerized Local Expenditure Accounts and Reports) has been fully implemented and utilized for fiscal analysis, and as an aid for resource use and allocation.

2. The use of contracts has been reviewed as an operational instrument for program and accomplishments. More appropriate categories have been used for some ongoing contracts and, wherever feasible, contract operations have been eliminated and the contract supported function returned to an NIH internal support area. A considerable saving of funds is anticipated. Two Laboratories were selected for program extension by use of the contract mechanisms. The implemented contracts directly extend the main

laboratory functions but represent only a small part of the overall laboratory program. The use of the contract mechanism has been reduced in two other laboratories.

3. Review and analysis for program planning for individual laboratories has been established on a semi-annual basis. The internal review serves a more direct operational purpose than does the program review by the Board of Scientific Counselors. An analysis of resource use by IR laboratories has been made for FY 72 by combing data obtained from CLEAR and the Extramural Program Computer Data System. Finding that the total professional man years used for IR programs far exceed the man years available from permanent positions attest to the appeal of the IR programs in attracting other professionals for on site participation in our programs.

Several organizational changes occurred during FY 73 that beneficially affected the Intramural Research Programs. A Chief has been appointed to direct the activities of the Surgical Neurology Branch. Three units now constitute the Branch: The Office of the Chief; the Section on Applied Research in Surgical Neurology; and the Section on Neuroradiology. The programs of the Branch place major emphasis on those areas of research that relate directly to the neurological disorders amenable to surgical procedures. Research on epilepsy, stroke, trauma, tumors and involuntary movements are of major importance in the current program. This reorganization represents a renewed orientation of the Branch to pursue more directly the unique research opportunities presented by patients with disorders amenable to treatment by neurosurgical techniques.

This fiscal year is the first full year of operation of the Developmental and Metabolic Neurology Branch. Reorganization took place early in the year and the Branch is now composed of four units: Office of the Chief; Section on Enzymology and Genetics; Section on Neurochemical Methodology; and Section on Neurological Development. The Branch is unusual in that it combines a basic research capability with a small clinical facility where the fundamental findings can be applied.

A program realignment was accomplished by the transfer of the Section on Nerve Regeneration from LNNS into the Laboratory of Neurochemistry, where it became the Section on Neural Development and Regeneration. This change was the natural and gradual evolvement of a collaborative effort between the receiving Laboratory and the transferred Section. It is believed that the transfer action will result in more coordinated programs in each of the involved laboratories.

To more accurately reflect the current program in the LNNS the Section on Comparative Neuropathology has been newly titled the Section on Cerebrovascular Pathology. A senior researcher has been recruited to expand tissue culture studies of LNNS.

During the past year the Central Nervous System Studies Branch was transferred from the Collaborative and Field Research area to the Intramural Research Program. Since the Branch has little direct clinical responsibilities and is mainly a laboratory operation, the unit is now called a Laboratory. In its new organizational setting the laboratory continues to elucidate the causes and pathogenesis of subacute and chronic degenerative disorders of the central nervous system. Multiple sclerosis, amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease and other presenile dementias remain a major focus. Continued study of patients with progressive dementia has provided the surprising demonstration that even the heredofamilial form of Jakob-Creutzfeldt disease is transmissible and of virus etiology. This demonstrates for the first time in man that disease with an apparent genetic determination may be caused by a slowly acting virus. The finding that non-inflammatory, chronic degenerative or even heredofamilial disorders could be of virus etiology has greatly stimulated the search for a possible virus etiology in the demyelinating and sclerosing central nervous system diseases and the chronic encephalitides. A sero-epidemiological approach to the study of multiple sclerosis has convinced the scientists of this laboratory that at least some patients suffer from a delayed and slow virus infection, probably with the measles virus. The search for such antigens in brain tissue progresses.

A group of atypical viruses has been found to cause the subacute spongiform virus encephalopathies (Kuru, Jakob-Creutzfeld, scrapie and transmissible mink encephalopathy. These viruses are unlike any others known to microbiologists. The laboratory has found that the atypical viruses are unusually resistant to ultraviolet and ionizing radiation, heat, proteases, nucleases and to formaldehyde. The atypical viruses have now been partially purified and successfully concentrated. Characterization of their physical, chemical and structural properties are underway. These viruses are believed to be composed of small units of infectious nucleic acids strongly bound to fragments of plasma membranes. Because of the immense importance to general microbiology to understand these and similar infectious agents, the laboratory exerts a major effort in this direction.

The Laboratory of the Central Nervous System Studies continues to focus its attention on long-term studies of the human biology of the many vanishing primitive societies. The studies are multidimensional but focus primarily on neurological disorders with an emphasis of virus and genetic studies. This approach offers unique and unusual opportunities for medical research not available by other approaches. The details of these innovative activities can be found in the summary of the Laboratory activities and in the individual project reports.

As a result of forming the Developmental and Metabolic Neurology Branch by combining two units to bring clinical and basic research together, some considerable advance has been made toward understanding of the lipidosis. The enzyme ceramidetrihexosidase, lacking in the tissues and organs of patients afflicted with Fabry's disease, has been purified. In two patients the abnormally high levels of the accumulating glycolipid in the plasma was significantly lowered by the administration of the enzyme. This finding provides an encouraging sign for the success of enzyme replacement therapy for patients with certain heritable metabolic disorders. The DMNB has also developed a procedure for the purification of glucocerebrosidase which is the enzyme missing in patients with Gaucher's disease. The laboratory is now in position to scale up the procedure to obtain more enzyme and then assess the efficacy of its administration to patients. DMNB also has demonstrated the reliability of the prenatal diagnosis of Gaucher's, Niemann-Pick, Tay-Sachs and Fabry's diseases. The Branch serves as a frequently consulted genetic counseling center and because of unavailability elsewhere provides diagnostic service for requesting physicians. This Branch has described a newly discovered myelin-specific glycoprotein. The metabolism of the new myelin component is deranged in mice which exhibit symptoms related to myelin malfunction. Work is in progress to determine the precise role of this substance in the development of the myelin sheath and to examine its quantity and composition in some of the demyelinating disorders of human beings such as multiple sclerosis.

Other noteworthy advances in chemistry were made during the year in the Laboratory of Neurochemistry. For the first time the $M_g$-dependent, Na-K-activated ATPase has been obtained in a stable form suitable for chromatographic, electrophoretic, structural and kinetic studies. This program appears to be at the threshold of elucidating the enzymological mechanisms responsible for the transduction of cellular energy into cationic transport across the cell membrane. This Laboratory has also for the first time provided detailed biochemical evidence to support the physiological concepts of molecular asymmetry of plasma membranes. These findings give promise of major advances in knowledge of the functional organization of cellular membranes. During the past year, the Laboratory of Neurochemistry has been able to assign a new function to astroglia. These cells, at least in culture, mediate the inward transport of chloride. The uptake of chloride by the astrocytes is inhibited by acetazolamide, a finding that may implicate the enzyme carbonic anhydrase as a mediator of chloride transport. The studies suggest strongly that the astrocyte is a key element in the fluid and ionic equilibrium of the central nervous system.

Progress also has been made in other IR Laboratories in understanding the biochemistry and neurochemistry of nervous system function and dysfunction. The Laboratory of Neuropathology and Neuroanatomical Sciences has demonstrated that the transport of glucose is significantly facilitated in brain subjected to ischemia. Hypoxia, hypercapnia and osmotic damage also affect glucose transport. Since complications in stroke involve hypoxia and hypercapnia these studies are relevant to clinical conditions. Also of importance are the studies from this Laboratory which reveal a drop in the level of serotonin, dopamine and norepinepherine in the area of brain infarction. Shown also was that after cold injury serotonin accumulates in damaged blood vessels. A study of the nature and dynamics of brain edema found that accumulation of abnormal fluid develops gradually between three and eighteen hours after the ischemic insult.

Two other areas of investigation in LNNS are directed toward the solution of major neurological problems. A major study of myelination and demyelination processes has led to the development of a system to test the effects of substances such as antibodies and sensitized cells thought to be associated with the demyelination which occurs in multiple sclerosis. The other study approaches the problem of paraplegia at a fundamental level and demonstrated that neurons can survive transplantation provided the immune response is inhibited and genetic histocompatibility differences are avoided. The transplanted neurons are capable of regenerating their severed processes and reestablishing a functional relationship with other cells. There is an ongoing major attempt to determine how to prevent regenerating axons from establishing improper connections while at the same time permitting them to establish functionally appropriate ones.

The work of the Laboratory of Molecular Biology produces information fundamental to our understanding of the nervous system at the cellular and molecular level. The research with bacteria has yielded many new findings which should be applicable to mammalian cells since their nucleic acids, membranes and metabolic pathways are similar to those of bacteria. It has been found that both in bacteria and cultered cells ATP is not required for the transport of most molecules. The transport studies led to the knowledge that the lipophilic acids, including the antimicrobial food additives, inhibit amino acid transport. An additional interesting finding was that the same substances also inhibit cultures of human cells. Two other findings were surprising, certain viruses would not grow in human cells when the lipophilic acids were present and the short chain acids altered the morphology of some epithelial-like cells. Those unexpected findings contribute appreciably to knowledge about how the food additives in use affect cells, and could lead to the development and use of more effective and safer substances. This Laboratory continues major efforts in studying the control of DNA and RNA synthesis, virus replication, and the genetic control of cell differentiation. The studies are of great fundamental importance in unraveling the mysteries of human cell function at the cellular and molecular level. The research findings are too detailed to be appropriately presented in this summary and can be referred to in the Laboratory reports.

The basic mechanism of nerve excitation and the transfer of information between cells are the most important properties of nerve tissue and possibly of existence itself. The Intramural Research Program therefore conducts a broad program in several laboratories that exhaustively pursues many approaches to understanding excitation and information transfer. In the Laboratory of Biophysics the focus of effort is the study of molecular events which are specifically coupled to nerve excitation and the generation of the action potential. In collaboration with the Laboratory of Neurochemistry, the biophysics group has isolated and characterized a Na$^+$- specific ionophore from Na-K-ATPase obtained from eel electoplox or beef kidney. The ionophore produces a voltage dependency in artificial bilayer membranes to that found in the nerve membrane. The final molecular characterizations of this substance would have heuristic implications for the understanding of cell excitation. Other work with ATPase has explained how ouabain manages to inhibit ATPase. The accumulation of K$^+$ in perineuronal spaces has been studied because this follows repetitive activity in the central nervous system. Since epileptiform discharges and other neural activities have been explained on the basis of K$^+$ accumulation, it is necessary to understand this phenomenon. By taking into account the K$^+$ data, the researchers have been able to modify the Hodgkin-Huxley equations to predict electrical phenomena observed during spike trains. A model of a membrane has been developed that allows work on the kinetics of a single membrane channel. It is believed that the ultimate of this new approach will lead to equations derived from molecular principles to replace the arbitrarily derived emperical equation of Hodgkin and Huxley.

The Laboratory of Neural Control also carries out research aimed at understanding the basic mechanisms of nerve cell activity. The group assumes the responsibility of applying the knowledge gained from the basic research to the development of prosthetic aids for the handicapped. The task is multifaceted and formidable. Because no method has been found for stimulating activity in nerve cells superior to the application of electrical fields through electrodes, emphasis is placed on research which leads to the design and use of improved electrodes. Together with the NIH-BEIB, the Laboratory has conceived and developed a microelectrode introducer which floats on air and follows the movements of the brain. The device may make possible the study of single nerve cell activity during brain surgery performed for the benefit of human beings. A new capacitor stimulating electrode is also under test. Its special value is that it does not produce deleterious electrochemical reaction with neural tissue. A newly developed groove electrode array for recording from single nerve fibers is ready for implantation tests. The studies from this Laboratory on spinal cord and cortical control systems have continued to produce results of fundamental importance for progress in the development of neural prostheses. Such results are imperative if success is to come in making either ingoing or outgoing connections to the central nervous system. The Laboratory continues to conduct successfully a neuroprostheses program supported by contracts which are dependent upon and coordinated with its internal research efforts.

The Neurophysiology Laboratory also investigates interactions between nerve cells. A major ongoing effort is the study of the neural organization of the retina which has led to a satisfactory description of synaptic contacts and electrical junctions between cells. This work has clarified the function of the horizontal cells in relation to the light receptors and has explained why the receptors respond electrically with opposite polarity for different distributions of retinal illumination. By means of microspectrophotometry, the Laboratory has been able to measure the absorption maximum of the pigments in single rods or cones and found that cones containing pigments of different colors are structurally different. Neurophysiological studies on invertebrate systems have identified the membrane mechanisms underlying rhythmicity of pacemaker neurons. The same mechanisms may be the basis for other rhythmical activities in heart or smooth muscle and thus of wide general interest.

Experiments performed in the Laboratory of Experimental Neurology support the concept that the propagation of focal paroxysmal activity seen in epilepsy shifts from a subcortical to cortical location as the animal matures. Anatomical findings from maturing animals also support the concept. This Laboratory has continued its work on x-irradiation damage on the central nervous system on monkeys. Cortical areas exposed to sufficient x-rays show proliferative and degenerative changes after delays of four or five months. The long-term structural changes included periodic brain swelling and retrograde transsynaptic degeneration within the visual system. A breakdown of the blood brain barrier accompanied the radiation lesion with loss of serum fluid into the brain parenchyma.

The pathophysiology and pathogenesis of perinatal brain damage has been clarified by the work of the Laboratory of Perinatal Physiology. The levels of oxygen deprivation producing malfunction or injury have been defined. The earliest change that follows severe oxygen deprivation in term-fetuses of monkeys is a dramatic shift of electrolytes and fluid between the various spaces of the brain. Water content increases, the brain swells, capillary circulation declines and the brain tissues necrose. These studies have also shown that episodes of fetal bradycardia which follow uterine contractions are indicative of fetal asphyxia. The administration of prostaglandins and adrenergic blocking agents to the mother may adversely affect the fetus, usually producing asphyxia. This knowledge is of importance since the prostaglandins have been proposed for use to induce labor and the blocking agents are increasingly used in pregnant women for control of hypertension. Another study has shown that primates surviving a 15-30 minute period of severe hypotension induced by drugs have undamaged brains. The biomedical studies of the Laboratory emphasize brain glycogen changes in response to metabolic or other challenges. A surprising finding was that during starvation brain glycogen increases while the glycogen of other organs is depleted.

The Surgical Neurology Branch orients its research to study the form, function and diseases of the human nervous system in relation to the ameliorative effects of neurosurgery. Using cases in which the pathology has shown complete lenticulothalamic or brachium conjunctivum destruction, a study has been made of retrograde degeneration of the dentate nucleus. Thirty to fifty percent of the dentate efferents were found to enter the human thalamus. Anatomical studies in nonhuman primates have indicated the existence of major connections between the anterior insula and the "arcuate nucleus" located immediately anteroinferior to the centrum medianum. Within its area of clinical studies the Branch investigates speech, memory and cognitive functions. Analysis of amytal testing for lateralization of speech indicated that early left hemisphere damage and relocation of speech to the right hemisphere results in a lower quality of intellect. Also discovered was that the right side of the cortex may have a more active function in extrastriate visual perception. By comparing parietal to temporal lesions using several memory tests, it was found that the parietal lobes provided visual memory while memory in temporal lobes related to auditory functions. Both basic and clinical aspects of tumors of the CNS system are investigated. Three glycoproteins have been extracted from glioma tissue in higher than control concentrations. The finding is of importance since these substances could be specific tumor antigens of use in therapeutic approaches. A study is ongoing to determine whether immunotherapy might complement or replace chemotherapy for brain tumors. The Branch continues its pursuit of diagnostic methods of benefit in relation to diseases of the CNS. Radioisotope angiography of the spinal cord has proved to be a simple and safe method of observing hemangioblastomas. Studies have begun to evaluate the use of computerized axial tomography of the x-ray absorption of the brain for diagnostic and experimental purposes. A new tomographic device, the EMI-scanner, may prove to be the first practical noninvasive method for quantitatively measuring responses to tumor therapy. Vascular disease of the CNS is an area of major importance for investigation within the Branch. A nonhuman primate stroke model has been developed that is capable of consistently inducing experimental infarctions. Nuclear magnetic resonance techniques are being developed to extend the study of regional blood flow in man.

A strong forward thrust to understand and treat neurological disease which involves the muscular system continues in the Medical Neurology Branch. The explanation proposed by this group which attributes Duchenne's type muscular dystrophy to ischemia mechanisms on the arterial side of the vascular network has been explored now by other groups. An improved animal model developed in the Laboratory is in use to screen for prophylactic drugs which might effectively prevent the ischemia. A cluster of histochemical techniques has been developed and used to identify carriers of Duchenne's dystrophy. These studies are of importance because they provide hope for a therapeutic advance for this baffling dystrophic disease.

Myotonia and paramyotonia congenita were studied histochemically and the evidence indicates a neurogenic basis. Other investigators have confirmed the finding of this Branch that immunoglobulins are deposited in the blood vessels of patients with the Polymyositis-dermatomyositis complex. The treatment introduced here, high-single-dose-alternate-day prednisone, has also been found by others to be the best available treatment for this disease. This type of prednisone treatment continues to be beneficial for most patients with myasthenia gravis and has now been adopted and confirmed by others. Thymus cells taken from some myasthenia patients and grown in culture have produced a substance which inhibits nerve-muscle transmission.

The Branch has described a new disorder, family sensory polyneuropathy with elevated serum IgT. A dysneural neuropathy secondary to the administration of the drug dapsone was identified. The Branch also described (with NEI) an acquired pupillary dysautonomia that is usually overlooked but is a frequent accompaniment of idiopathic non-familial neuropathy.

The motor neuron diseases continue to be an important area for study. The Medical Neurology Branch has shown that lower motor neurons are reversibly affected in hyperparathyroidism. Studies on central core disease have indicated that it is likely a neurogenic disorder. An early atrophy of type II muscle fibers has been found in patients with non-neurologic localized cancer. A new syndrome of myoclonus epilepsia has been elucidated. A case of subacute sclerosing panencephalitis has been found in one of a pair of identical twins, an important finding in relation to the genetic aspects of the disease. Although the prime concern of this Branch is the clinical neurological disorders, a not insignificant contribution is their development of ways to apply basic research techniques to the neurological problems to be solved.

A major interest in the Intramural Research Program is a study of epileptogenic mechanisms. The Electroencephalography Branch, in cooperation with the Surgical Neurology Branch, has developed a method which allows the entire area of exposed cortex to be observed for changes in oxidative metabolism in relation to the corresponding electrographic phenomena. The method is based on the non-invasive fluorometric monitoring of NADH changes in the surface layers of the cortex by use of a television camera and image intensifier. The method can be of importance since it can rapidly gather data from the human cortex when time to do so is limited. The project dealing with the long-range follow up studies on the surgical treatment of patients with seizure disorders has continued. The part of the study dealing with technique, indication, limitation and interpretation has been completed. The final results of the study will be of practical value not only for assessment of the usefulness of surgical treatment in focal epilepsy but also in the selection of those cases which are likely to benefit from such therapy. The Branch has begun a new study on epileptic patients with evidence of involvement of the occipital cortex. The data provides information on the localizing and lateralizing value of the signs and symptoms in the ictal episodes of such patients and thus can be of significance in differential diagnosis. The cellular mechanisms which are possibly subservient to the epileptogenic processes are investigated. These studies provide information on the basic pathophysiological mechanisms of epileptogenesis. The clinical diagnostic service that this Branch provides for all patients in the Clinical Center remains an important function of the unit and uses a considerable amount of the manpower available.

During the past year the Intramural Research group conducted 144 active projects. Thirty-one new projects were begun. Nine projects were completed and 15 were terminated. Of the 144 projects active this year, twenty-five were carried on in collaboration with one or more outside research Institutes and twenty-eight were active in cooperation with other NIH units. Eighteen IR projects were in dual collaboration with NIH and other Laboratories. Nine scientists from the Intramural Program served as Project Officers on projects carried out in foreign countries under support by the PL 480 programs. Intramural has had an average of 25 foreign scientists working in its programs during the year, either in the guest worker or visiting category. These extensive interactions with other research efforts and scientists attest to the marked influence of the Intramural Program upon both the domestic and foreign research communities.

# Annual Report of the Section on Technical Development

# National Institute of Mental Health

# National Institute of Neurological Diseases and Stroke

July 1, 1972 - June 30, 1973

James S. Bryan, Chief,

During the past 12 months, the Section on Technical Development (STD) continued to fulfill its traditional role of support of NIMH and NINDS research efforts. Continuing assistance was made available in shop help, special purpose instruments, instrumentation systems, and assistance in computer oriented research and digital instrumentation.

The Section continues as before to support three laboratory computers for general use, as well as the medium-size SEL 810B installation in Bldg. 10. The 810B installation is now working well, supporting as many as four simultaneous users in on-line and real-time research applications. Hardware and software support for this installation has tapered off now to a routine level of one man year for the programmer operator and one-half man year for hardware maintenance and new interface requirements. Table I gives a breakdown of laboratory computer usage by Institute. Table II shows current weekly utilization of the SEL 810B by individual sections. Table III shows a breakdown of overall activity by STD.

As usual, much research within the Section has involved collaboration with laboratories in NIMH and NINDS, and is, therefore, reported as part of the projects in the laboratories involved. Exceptions to the foregoing include our continuing efforts to develop equipment to measure motor dysfunction associated with dystonia and Parkinson's disease, and a short term project to develop a sensory aid for the blind using a binaural presentation of visual space.

The studies of Parkinsonism have progressed to routine testing of selected patients supplied by the Section on Experimental Therapeutics, Laboratory of Clinical Science, NIMH. An initial description of the testing equipment is being presented at the Rocky Mountain Bioengineering Conference in May 1973, and patient testing is going forward in an attempt to investigate an hypothesis involving a multiple feedback model for Parkinson tremor.

The short-term project to produce a visual aid for the blind began as a part-time effort on the part of a post doctoral engineer occupying a two year rotating Commissioned Corps position. This man's personal decision to leave the National Institutes of Health after one year to enter medical school caused us to accelerate the project in order to achieve some useful outcome. This accelerated effort has caused the STD project total in Table III to be substantially higher than usual for this year. The device appears to offer substantially better subjective resolution than some similar devices such as the Bach-y-Rita tactile stimulator at the expense of increased time to present the complete "visual" field -- three to five seconds depending on content. Because of the time factor, the device seems not to be attractive for navigational guidance but may prove to be a useful vocational aid.

A total of 151 projects were completed in the last year. A large portion of these consisted of routine fabrication of various chambers, baths, electrode holders, and jigs for experimental use as well as many small electronic devices, and systems.

Major systems included:

(1) Completion of a large-animal telemetry system with one-half mile range for study of sleep patterns in free ranging wild animals - Laboratory of Clinical Psychobiology, NIMH. Last report from Africa is that the system is working well with wildebeests up to 3/4 mile range for periods up to 11 days.

(2) Expansion of the Poolesville rat colony data acquisition system - Laboratory of Brain Evolution and Behavior, NIMH.

(3) Programmed display and response system for family interaction studies - Adult Psychiatry Branch, NIMH (in process).

(4) Telemetry system for monitoring patient activity - Laboratory of Clinical Psychopharmacology, SMRC, NIMH (in process).

## TABLE I

## LABORATORY COMPUTER UTILIZATION

| | NIMH | NINDS | NICHD | TOTAL HOURS |
|---|---|---|---|---|
| Classic LINC | 100% | 0 | 0 | 1490 |
| MicroLINC | 69% | 31% | 0 | 1931 |
| PDP-12 | 32% | 54% | 14% | 1521 |

## TABLE II

## ESTIMATED CURRENT SEL 810B UTILIZATION

| | Hours per week* |
|---|---|
| Laboratory of Psychology, NIMH | |
| Section on Higher Thought Processes | 10 |
| Unit of Psychophysiology | 30 |
| Section on Personality | 0.5 |
| Section on Perception | 5 |
| Adult Psychiatry Branch, NIMH | |
| Section on Twin and Sibling Studies | 0.5 |
| Section on Perceptual and Cognitive Studies | 30 |
| Laboratory of Clinical Psychobiology, NIMH | |
| Section on Comparative Studies | 2 |
| System Development | 8 |
| | 86* |

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*Timeshared terminal hours, hence more than 40 per week.

## TABLE III

## APPORTIONMENT OF EFFORT

## ENGINEERING & FABRICATION TIME

## MAJOR USERS

| LABORATORY OR BRANCH | HOURS | PERCENT |
|---|---|---|
| Brain Evolution & Behavior, NIMH | 3965 | 13.63 |
| Psychology, NIMH | 3873 | 13.31 |
| Technical Development, NIMH/NINDS | 3574 | 12.29 |
| NIMH 810B Computer | 3227 | 11.09 |
| Clinical Psychobiology, NIMH | 2625 | 9.02 |
| Neurophysiology, NINDS | 1810 | 6.22 |
| Behavioral Biology, NICHD | 1613 | 5.54 |
| Adult Psychiatry, NIMH | 1586 | 5.45 |
| Biophysics, NINDS | 1122 | 3.85 |
| Office of the Director, NINDS | 778 | 2.67 |
| Neurophysiology, NIMH | 759 | 2.61 |
| Neurobiology, NIMH | 478 | 1.64 |
| Clinical Science, NIMH | 424 | 1.45 |
| General & Comparative Biochemistry, NIMH | 412 | 1.41 |
| Neuropathology & Neuroanatomical Sciences, NINDS | 401 | 1.38 |
| Experimental Neurology, NINDS | 397 | 1.365 |
| Molecular Biology, NINDS | 386 | 1.327 |
| Neurochemistry, NINDS | 340 | 1.169 |
| Socio-environmental Studies, NIMH | 335 | 1.152 |
| Cerebral Metabolism, NIMH | 328 | 1.128 |
| All other NINDS Labs | 393 | 1.351 |
| All other NIMH Labs | 251 | .863 |
| NIMH (Total) | 20,050 | 68.95 |
| NINDS (Total) | 7,414 | 25.52 |
| NICHD (Total) | 1,613 | 5.53 |
| TOTAL* | 29,077 | 100.0 |

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*Not included in the foregoing totals or in the breakdown by laboratory are 1109 hours spent in routine maintenance internal to the Section, and the time of the Section Chief.

# ANNUAL REPORT
## July 1, 1972 through June 30, 1973
## Report of Acting Clinical Director
## National Institute of Neurological Diseases and Stroke

J. M. Van Buren, M. D.

The Branches of A) Electroencephalography and Clinical Neurophysiology, B) Medical Neurology, C) Surgical Neurology, and D) Developmental and Metabolic Neurology carry on the clinical and clinically applied studies of Intramural Research, NINDS.

### A. Electroencephalography and Clinical Neurophysiology Branch

This Branch has continued to provide a clinical-diagnostic service for all in- and out-patients of the Clinical Center referred for EEG examination. Such referrals are either part of the routine diagnostic work-up, or control of patients from the various Institutes, or are parts of investigative projects originating outside of this Branch. As in previous years this form of activity has accounted for a considerable portion of the overall activity of the Branch, requiring a total of over five man-years. In the last twelve months 1,303 EEG tracings have been obtained and interpreted on the basis of corresponding referrals. The largest number of these have come from NINDS, and predominantly from the Surgical Neurology Branch. All other Institutes combined have accounted for one-third of the referrals, with NCI and NIMH being the largest source after NINDS. A certain number of examinations have been provided directly in the Ward for patients in ICU. As in the past the Branch has collaborated very closely with the Surgical Neurology Branch in both diagnostic service and clinical investigative projects. About 30 tracings were obtained from patients in which electrodes had been chronically implanted for therapeutic purposes in the case of abnormal movement disorders and for diagnostic localization purposes in the case of seizure disorders. Direct recordings from the exposed cortex have been obtained on 12 additional patients in the course of surgical exposure of the brain for the treatment of epilepsy. The latter has continued to represent the focus of research interest of the Branch. A major effort has presently begun to evaluate all of the pre- and post-operative data on the large number of patients who have undergone cortical excision for the management of their seizure disorder. Such a study should provide useful information for the establishment of prognostic criteria and for the selection of the most suitable candidates for surgical treatment. Other research projects carried out on patients include a correlative study between EEG and clinical seizure patterns in epileptic subjects with lesions in the occipital lobe and a monitoring of intracellular redox changes in the human cortex.

### B. Medical Neurology Branch

Introduction: The clinical activities involved 269 patient admissions for a total of 5,601 patient days. There were 648 outpatient visits. Neurological consultations were provided to 504 patients on other services of the Clinical

Center. There were 293 muscle biopsies processed histochemically. Thirteen research communications were published, 18 are in press, and 19 were presented to meetings. The one-year approved residency training program in neurology has continued. About 15 physicians and 10 technicians came as guest workers to learn our clinical investigative techniques. Some of the results of our clinical studies are the following. (I = in progress, P = in press, R = reported or published.)

1. Myasthenia Gravis (MG)

High-single-dose alternate-day prednisone (HSDAD-Pred), introduced to the treatment of this disease by us, continues to be effective in 17 of our 19 cases treated as long as 6 yrs. in a child and 3 yrs. in an adult (R, P,I). Our two non-responders were females in the menstruating age group. The beneficial response to HSDAD-Pred has been confirmed by other groups. The binding site of anti-muscle factor characteristic of MG was localized with immunohistochemistry-electronmicroscopy to the sarcoplasmic reticulum but only at the I-band level, showing a chemical specialization of this part of the reticulum (P). Suspected from clinical studies in MG, an adverse interaction between corticosteroid and anticholinesterase drugs has been demonstrated in an animal nerve-muscle test preparation (P).

2. Inherited Myopathies

In the Duchenne type of muscular dystrophy (MD) impaired uptake of serotonin into platelets was demonstrated (R) with urine and spinal fluid levels of various catecholamines and indolamines being normal (R). In our putative model of Duchenne MD, i.e. arterial-tree ischemia induced in the rat, a rise of serum "muscle enzymes" with the administered vaso-active amine was shown, providing a further parallel with Duchenne MD and basis for screening drugs potentially useful clinically (R). Further clinical and laboratory evidence continues to suggest that several erstwhile myopathies, e.g., myotonic atrophy (myotonic dystrophy), myotonia congenita, central core disease, and type-I-fiber-hypotrophy-~~with~~-central-nuclei, actually are more likely to be based on a subtle partial abnormality of the neural influence on muscle (R,P,I). In muscle phosphorylase deficiency (McArdle's disease), it was demonstrated that, rather than irrevocable genetic absence of the enzyme, phosphorylase activity can actually be provoked in muscle fibers regenerating in vivo and in vitro (R), providing a possible approach to treatment.

3. Polyneuropathy (Peripheral Neuropathy)(PN)

Immunoglobulin survey of a large group of presently idiopathic forms disclosed elevation of serum IgM in some, suggesting an immunologic pathogenesis (P). A new disorder was discovered in one family, elevation of serum IgA, shown to be due to IgA overproduction in the gut cells (P). Treatment of some cases of idiopathic PN with HSDAD-Pred continues to be very successful and necessary, in some for as long as 8 years (I). Dapsone-induced PN was identified (R). The PN of xeroderma pigmentosa was identified and its possible relation to the previously known defective DNA repair mechanism speculated upon (P).

### 4. Hypokalemic Periodic Paralysis

The treatment we introduced, acetazolamide, continued to be effective in the majority, in some for as long as 7 years (R,I).

### 5. Amyotrophic Lateral Sclerosis (ALS)

Different approaches continue to fail to disclose the cause. For example, the toxic effect of ALS serum on cultured motor neurons reported from other centers is not demonstrable in our laboratory (I).

### 6. Hyperparathyroidism

In 16 patients the major neurologic involvement has been shown to be of lower motor neurons, sometimes with bulbar lesions and brisk reflexes giving an ALS-like syndrome; nearly all patients reverted toward normal following surgical treatment of their hyperparathyroidism (P,I).

### 7. Epilepsy

A new syndrome of myoclonus epilepsy and limb-girdle dystrophy with "ragged-red fibers" and impaired oxidation of butyrate by muscle slices has been identified (P).

### 8. Cancer

Because viral-induced experimental brain tumor is a likely model for the etiology of at least some human brain tumors, the factors influencing the cell type of the experimental tumors were elucidated (R). The muscle wasting as a distant effect of various types of cancer was studied by a combined electromyographic-histochemical approach and found to be probably based on a subtle altered neural influence on muscle (I).

### 9. Clinical Investigative Techniques

Open biopsy electromyography, done in the operating room during muscle biopsy, was developed as the first method to allow direct correlation in humans of physiology during voluntary effort with histochemistry of muscle fibers; it led to the identification of the type I unit pattern as a slow sustained rhythmic discharge during slight effort and the type II unit pattern as limited bursts of rapid discharges only during greater effort (R). In tissue culture excellent and reproducible growth of human muscle biopsied from adults or children was achieved; the growth characteristics and ultrastructure of normal human muscle in culture were reported (R). No abnormality in rate or pattern of growth properties was noted in muscle of several neuromuscular diseases cultured (I).

## C. Surgical Neurology Branch

Introduction: Clinical activities included 610 outpatient visits and 236 patient admissions for a total of 6,085 patient days on the service.

Ninety-eight operations were carried out in the following categories:

| | |
|---|---|
| Craniotomy - tumors | 22 |
| " - cerebral seizures | 9 |
| Electrode implant by stereotaxic method | 9 |
| Laminectomy - tumors | 6 |
| Transsphenoidal hypophysectomy | 5 |
| Laminectomy - AVM | 4 |
| Craniotomy - brain biopsy | 3 |
| Laminectomy - cervical fusion | 2 |
| " - cordotomy | 2 |
| Craniotomy - removal bone tumor & cranioplasty | 1 |
| Laminectomy - disc disease | 1 |
| Trephination (3) - subdural hematoma | 1 |
| Total Major Operations | 65 |
| | |
| Shunts | 24 |
| Bone biopsy - temporal | 2 |
| Carpal tunnel lysis | 2 |
| Debridement of wound | 2 |
| Depth electrode removal | 2 |
| Cranioplasty | 1 |
| Total Minor Operations | 33 |
| | |
| Grand Total | 98 |

Branch activity continues to pursue studies in 1) Tumors of the CNS, 2) Vascular Disease of the CNS, 3) Epilepsy, 4) Dyskinesiaa, 5) Speech, Memory and Cognitive Function in Man, and 6) Effects of Trauma on the CNS.

1. Tumors of the CNS

Glioma tissue has provided three glycoproteins which are being characteriz as possible tumor specific antigens.

The finding that patients with grade III and IV gliomas showed decreased cellular immunity to DNCB has suggested that intracerebral PPD after intradermal BCG might enhance nonspecific cellular immune response and thus prove to be a useful adjunct to chemotherapy. Initial trials in monkey showed no serious consequences, so that trial in human therapy is planned.

Several new diagnostic techniques have appeared. Application of cisternography to study of cerebral flow patterns in monkey have provided a baseline useful in location of cerebral tumors. Radioisotope angiography of the spinal cord by I.V. injection has proved safe and useful in demonstrating vascular malformations. Studies of specific X-ray absorption factors in tissue are being undertaken with the newly developed EMI-scanner which promises to be a revolutionary diagnostic device.

2. Vascular Disease of the CNS

Basic microangiographic studies continue on the spinal cord of human fetuses and children, ~~with~~ completion of this fundamental work. On this background postradiation myelitis and postangiographic myelopathy are being studied experimentally.

The primate "stroke" model has provided a basis for study of effects of $CO_2$ and $O_2$ variations and will be used for testing a proposed nuclear magnetic resonance method for estimation of regional blood flow in the brain. If successful the method is important since it is noninvasive and infinitely repeatable.

3. Epilepsy

Study continues on the practical application of NADH assay on cerebral cortex exposed at craniotomy. A technological advance incorporating TV imaging and color transformation of florescence brightness values promises a device capable of topographical resolution and real-time analysis.

A comprehensive analysis of over 300 surgically treated patients with seizures and over 3 years of follow-up continues with development of a system for punch card data storage.

4. Dyskinesias

Completion of a review of motor testing techniques has led to formulation of plans for improved methods in this difficult field.

Collection of specimens for clinico-anatomical studies in dyskinesias continues. The relationships between the dentate nucleus and the diencephalon in man has been started with investigations to date centered on study of normal variations of this nucleus. As usual what is considered as "well known" has proved to be unexplored in human specimens.

5. Speech, Memory and Cognitive Function in Man

A variety of studies continue making use of clinical opportunities including carotid amytal injection, cortical and depth stimulation and cerebral ablation. The thrust of the endeavor has centered upon further characterization and quantitation of the areas such as temporal-parietal functional differences, impairment of consciousness associated with fronto-striatal stimulation, relationship of memory and speech errors in dysphasic responses to stimulation and variations in the recognition of material presented peripherally in the visual fields tachistoscopically after focal cerebral resection outside the primary visual cortex.

6. Effects of Trauma on the CNS

Time has been required for the fabrication of a special Head Accelerating Device (HAD) and finding a suitable situation for continuing these experiments. Studies have continued on the intracranial Pressure-Volume curve demonstrating that it is extremely rate sensitive. Thus at low rates of volume increase the vasopressor response is not elicited.

## D. Developmental and Metabolic Neurology Branch

The clinical activity of the Branch included 65 inpatient admissions with 1460 patient hospital days and 93 outpatient visits.

This new Branch was formed in July 1972 by combining the Section on Child Neurology, formerly part of SNB, and the Section on Lipid Chemistry, formerly part of LNC. The following are the main objectives of the new Branch: 1) Development of replacement therapy in certain disorders of inborn errors of metabolism, especially in mucopolysaccharidoses and lipidoses, utilizing purified specific enzymes and blood products with high content of enzymatic activity. 2) Study of the mechanisms of therapeutic effects of the high fat diet, progesterone and certain anticonvulsant drugs in epilepsy. 3) Clinical and etiologic delineation of certain genetic disorders with particular emphasis on mental retardation.

Using the combined facilities of the new Branch, it has been possible to infuse, for the first time, missing enzymes in three different diseases: Tay-Sachs' disease, Fabry's disease, and preparations are being made to infuse β-gluco-cerebrosidase in Gaucher's disease. Several patients with mucopolysaccharidoses received blood products replacement therapy. Parts of studies on delineation of certain genetic disorders, epilepsy and prenatal development were completed and published or are in press.

Serial No. NDS(I)-66-ODIR 1460
1. Office of the Director of Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Speech habilitation of the congenitally deaf--Fundamental problems and suggested directions

Previous Serial Number: Same

Principal Investigator: Rosalind B. Marimont

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.1
Professional: 0.1
Other: 0.1

Project Description:

Objectives: To analyze the reasons for the general lack of success in speech habilitation of the congenitally deaf and to suggest areas of research which might contribute to progress.

Methods:

1. Review of literature.

2. Consultation with teachers of the deaf, and experts in allied fields--engineering, speech studies, and physiology, among others.

3. Analysis of problems.

Major Findings: The importance of interdisciplinary research, particularly the application of computer speech processing technology, stressed by this investigator since 1966, is becoming recognized. A program for research in many fields, presented in a paper "How can we teach the deaf to speak," hitherto unpublished was brought up to date and the paper accepted for publication in the A.G. Bell Association Quarterly Review, a new research journal to begin publishing soon.

Proposed Course of Project: To follow developments in the field and possibly resume active work at some later date.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68-ODIR 1554
1. Office of the Director of Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Pure and applied mathematics, with special attention to mathematical models of sensory systems and of research institutions

Previous Serial Number: Same

Principal Investigator: Rosalind B. Marimont

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.7
Professional: 0.7
Other: 0.0

Project Description:

Objectives: To conduct research in pure and applied mathematics, with special attention to mathematical models of sensory systems and also of scientific research institutions. The latter will be directed toward improving the scientific management decision making process (systems analysis).

Methods Employed: Mathematical Analysis

Major Findings: An economical, easily operated computerized system (CLEAR) to record cost data in a form suitable for mathematical analysis is feasible for a relatively small research group (about 250 employees). Regular reports to management are a useful byproduct of such a system and greatly simplify day-to-day decision making.

Comparison of detailed costs over two years of operation show wide differences among Laboratories and Branches, but a surprising constancy for each Laboratory and Branch for the two years.

The methods of matrix algebra in conjunction with computerized cost data greatly simplify changing the basis of resource allocation and analysis, as for example from analysis by organizational unit to analysis by program.

Proposed Course of Project: To study models already developed and determine their applicability to intramural research, and to improve these models or develop new ones.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70-ODIR 1789
1. Office of the Director of
Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The physical senses

Previous Serial Number: Same

Principal Investigator: Edith L.R. Corliss, Physicist

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.3 |
| Professional: | 0.3 |
| Other: | 0.0 |

Project Description:

Objectives: Preparation of a publication summarizing and interpreting experimental results on the properties of the normal human senses of sight, touch and hearing. These intact human senses are to be considered quantitatively somewhat by analogy to the description and evaluation of measuring instruments, i.e., which mechanisms are optimized, what resolution limits are obtained, over what dynamic range do they function?

Major Findings: Survey of the current literature has continued, in order to obtain and interpret current experimental data on the performance characteristics of the intact human senses of sight, touch and hearing.

What started out to be a minor revision and bringing up to date of a paper on the quantitative consequences of hearing loss, to assist the task force on hearing loss, turned out to result in a major reworking and extension of the material. The paper has passed through preliminary editorial review at the National Bureau of Standards and, what is hoped to be a final draft, is being prepared for submission to NINDS, with the aim of publication in an outside journal, probably the Journal of the Acoustical Society of America. It proved possible to set up quantitative predictions for the ability of persons with impaired hearing to recognize randomized word lists. These predictions agreed fairly well with experimental results. Thus it becomes possible to proceed from a quantitative knowledge of the performance of persons with unimpaired hearing to predict what will occur when the same tests are administered to individuals with well-specified diagnoses of hearing loss. Unfortunately, the simple threshold measurements of hearing conventionally used do not yield the necessary diagnostic data to distinguish among several types of hearing loss. The paper calls attention to the possibility of improved diagnosis of hearing loss by means of relatively simple suprathreshold tests. The most important of these are the difference limen for intensity, measured as a function of frequency and sensation level, and the masked threshold for pure tones in an intermediate level of noise background. (Perhaps 20-30 dB re threshold in the quiet). In the publication of this paper, it will be presented as a joint contribution of the NINDS and the National Bureau of Standards.

These new suprathreshold tests may be worth making in a clinical study for the fitting of hearing aids. They might prove helpful in estimating the degree of supplementation available to each individual in terms of his hearing loss.

A paper on the channel capacity of the ear as a function of frequency, an outgrowth of the speech recognition study, was presented at the Fall, 1972 Meeting of the Acoustical Society of America, and is being written up as a separate paper for journal publication.

A revised draft of the paper "The Recognition of Speech" was prepared after review comments and has been resubmitted for editorial review at the National Bureau of Standards.

Proposed Course of Project: The remaining independent, free-standing papers on which work has been done, namely, "Purely by Coincidence," "Interpretation of Concepts of the Critical Band" will be completed through editorial review. These will be reedited for inclusion in the latest draft of the section on hearing. In general, the preparation of free-standing papers has proved a slow and laborious process. It remains to be seen, from comparison of revised drafts with the original draft, how well this intermediate editorial process improves the ultimate quality of the writing in the book. Thus far, the writings have led to the development of new and original material that might otherwise not have been produced.

Honors and Awards: None

Publications: Corliss, E.L.R.: Remarks on "Fixed Scale for Absolute Pitch" (Letter to the Editor) J. Acoust. Soc. Am. 53: 1973.

Annual Report
July 1, 1972 through June 30, 1973
Medical Neurology Branch, IR
National Institute of Neurological Diseases and Stroke

W. King Engel, M.D.
Chief, Medical Neurology Branch

Introduction: An inter-related multidimensional attack on the chosen target diseases is emphasized in our application of basic research techniques to clinical neurologic problems. The current techniques consist of: histochemistry, clinical neurophysiology, biochemistry, immunology, electron microscopy, and tissue culture. In the human neurologic disorders studied, these techniques support thrusts to seek: (a) more precise morphologic, electrical, and chemical definition of the abnormalities; (b) separation of each disorder into more distinct and often new sub-forms; (c) specific or symptomatic treatment; and (d) induced animal models closely related to the human pathophysiologic states.

For the clinical investigations, 269 patients were admitted for a total of 5,601 patient days, and there were 648 outpatient visits. There were 293 muscle biopsies processed histochemically. The neurologists provided consultation on 394 patients of other departments in the Clinical Center, performing indicated biopsies, myelograms, pneumoencephalograms, and electromyograms. In the past year, 13 papers were published, 18 are in press, and 19 were presented to meetings. One staff member has devoted his time to serving as Acting Assistant Scientific Director, IR, NINDS.

The one-year approved residency training program in neurology has continued. Approximately 15 neurologists and other physicians and 10 technicians came this past year as guest workers to learn clinical research techniques in neurology, especially in neuromuscular diseases and the application of enzyme histochemistry thereto.

The Department of Neurology, Warsaw Medical Academy is continuing its collaborative research program in neuromuscular disease with us under the PL-480 program.

Muscular Dystrophies and Other Non-Inflammatory Myopathies: Our new hypothesis that the Duchenne type of muscular dystrophy (DMD) is likely to be caused by ischemia mechanisms on the arterial side of the intramuscular vascular tree is now being explored by other groups. We have extended our model (rat aorta ligation followed by vasoactive amine) to report that following each dose of serotonin the serum "muscle enzymes" rise, as in DMD. We are now using this improved model to screen for prophalactic drugs which might be effective clinically in preventing muscle ischemia. In DMD we have reported that although urine and CSF levels of various catechol and indol amines are normal, there is a decreased uptake and decreased content of serotonin in platelets (with NIMH). If a similar defect exists at arterial vessel sites in DMD muscle, vasoconstriction could be abnormally prolonged.

In clinically normal carriers of DMD, a cluster of histochemical reactions done on their muscle biopsies has proved very useful for carrier identification. In skeletal muscle phosphorylase deficiency (McArdle's disease), previously thought to be a complete genetic absence of that enzyme, we have reported that phosphorylase activity can be "revived" in muscle cells of those patients regenerating in vivo or in vitro, offering a new therapeutic possibility for the patients. A bizzare polysaccharide material has been found as the storage material in muscle in a rare type of cardio-skeletal myopathy. In myotonic atrophy (myotonic dystrophy), several lines of evidence continue to suggest to us that muscle involvement in this disease is not myopathic, but due mainly to an altered nerve influence on muscle (and therefore should not be listed in the dystrophy category). In myotonia congenita and paramyotonia congenita, we have now postulated a major neurogenic aspect to the pathogenesis, on the basis of histochemical evidence.

Polymyositis/Dermatomyositis Complex (PM/DM): Our finding of immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases, reported last year, has now been confirmed by others. This supported our earlier hypothesis that childhood PM/DM may be another form of blood vessel abnormality in muscle, but on the venous side. Since this and other immunologic abnormalities of PM/DM could in turn be caused by a viral infection, search for Australian antigen, circulating or bound to blood vessels or muscle cells, was made but proved completely negative (with CC). A model of viral myositis is being established. The method of treatment we introduced to this disease 5 1/2 years ago, high-single-dose-alternate-day prednisone (HSDAD-Pred), has continued to prove to be, in our ha (about 28 cases) and others, to be the single best available treatment for children and adults without or rarely with cancer. It has the greatest therapeutic benefit, fewest side-effects, and is easiest to manage.

Periodic Paralysis (PP): In the hypokalemic form of PP, the treatment we introduced, acetazolamide (Diamox), has continued to be the best prophylactic agent for preventing attacks and improving inter-attack weakness, and it is widely accepted as such. Two of our patients have been treated successfully for more than 7 years. The mechanism of acetazolamide benefit in hypokalemic PP remains unknown, since muscle does not contain carbonic anhydrase.

Other Myopathies: In one case of late-onset rod disease an associated unusual serum paraprotein, IgG/λ myeloma protein, has been found; intranuclear rods, identical histochemically and ultrastructurally to those of the cytoplasm, were also found in him, suggesting an origin from a contractile or other protein common to both nuclear and cytoplasmic sites. A new clinically delineated "levitated arms syndrome" has been described in 2 cases and the cause defined as iatrogenic intramuscular injections into the deltoids; one was successfully treated by surgical transection of fibrous bands.

Neuromuscular Junction Abnormalities: The treatment we introduced to myasthenia gravis (MG), HSDAD-Pred, has been confirmed and adopted by many other physicians. In our own series it continues to be extremely beneficial in the majority of cases, 17 of 19, and for as long as 7 years in a child and 3 years in an adult. Our only 2 non-responders were females in the menstruating age group. We have recently modified the treatment slightly by giving the single-dose 100 mg prednisone daily for the initial 2-3 weeks before converting to the alternate-day schedule, probably resulting in more rapid improvement. Anticholinesterase drugs are stopped prior to treatment and have not needed to be resumed. Suspected from clinical studies in MG, and adverse interaction between corticosteroid and anticholinesterase drugs has been demonstrated in an animal nerve-muscle test preparation. The binding site of the anti-muscle factor characteristic of MG (which we previously showed was an IgG and true antibody), was localized with immunohistochemistry-electron-microscopy to the sarcoplasmic reticulum but only at the I-band level; this shows chemical specialization of the reticulum of the I-band in contrast to that of the A-band. With immunofluorescence we have demonstrated that a supposed "neuron-specific" nuclear binding of MG patients, as reported by others, is actually non-specific and simply a general anti-nuclear activity well-known in some patients with MG and other "autoimmune" diseases. Tissue from thymuses of 5 of 8 MG patients during 24-48 hours in tissue culture liberated a substance(s) strongly inhibitory to an animal nerve-muscle test preparation.

Polyneuropathy (Peripheral Neuropathy) (PN): Immunoglobulin survey of a large group of cases of PN currently considered idiopathic disclosed elevation of serum IgM in some, suggesting an immunologic pathogenesis in them. A new disorder was discovered, family sensory PN with elevated serum IgA, shown to be due to IgA overproduction in the small bowel tissue -- on this basis a new hypothesis was introduced, one of possible familial susceptibility to infection in the gut tissue (in which there are excessive eosinophils) that causes through, or parallel to, the IgA overproduction a metabolic abnormality affecting sensory nerves. Our treatment of some cases of idiopathic PN with HSDAD-Pred continues to be very successful and necessary, e.g., for as long as 8 years in an adult and 5 1/2 years in a child. Correlative studies indicate that patients most likely to respond are dysschwannian in type (slow nerve conduction times), relapsing, with elevated CSF protein, but even some patients with a dysneuronal type of PN, non-relapsing, or normal CSF have responded. A dysneuronal neuropathy secondary to dapsone therapy was identified, with marked clinical improvement following discontinuation of the drug (with NCI). The PN of xeroderma pigmentosa was identified and its possible etiology speculated upon: it was hypothesized that the previously known defective DNA-repair mechanism might cause a shortened life in non-mitotic (neuronal) cells (enhanced neurologic "aging") because of inability to properly repair ordinarily banal neuronal DNA injuries (with NCI). Acquired pupillary dysautonomia (light-hyposensitive, tonic, mecolyl-hypersensitive) has been identified and shown to be a usually-overlooked but not infrequent (11 of 16 patients) accompaniment of idiopathic non-familial neuropathy, especially when there is a major sensory component (with NEI).

Motor Neuron Diseases: In amyotrophic lateral sclerosis (ALS), different approaches continue to fail to disclose the cause. For example, the "toxic effect" of ALS serum on cultured motor neurons, reported from another center, is not demonstrable in our laboratory. Benign focal amyotrophy has been described as a disorder simulating the lower motor neuron aspects of ALS at its onset but of spontaneously limited progression. In hyperparathyroidism we have shown that there is neurologic abnormality in nearly every case, the most common involvement being of lower motor neurons, sometimes with bulbar involvement, tongue tremors, and brisk reflexes giving an ALS-like syndrome, which reverts to or toward normal following surgical treatment of the hyperparathyroidism (with NIAMD). Further evidence was adduced by ourselves and others to support our earlier hypothesis that central core disease is more likely to be an abnormality of the neural influence on muscle than a myopathic disorder as originally proposed by others. On the basis of histochemistry and electronmicroscopy, three new disorders were described, (a) a new form of central core disease in which there is a "hot spot" of enzymatic activity and lipid droplets in the center of the core, (b) focal loss of cross-striations, possibly due to defective motor neuron influence on muscle, and (c) type I fiber smallness without central nuclei. A new form of the benign congenital hypotonia syndrome was described, consisting of marked type I muscle fiber predominance -- this was proposed to be caused by an in utero time-limited neuropathy affecting mainly the type I motor neurons. In patients with non-neurologic localized (non-metastatic) cancer before there is clinical evidence of anorexia, weight loss, or decreased daily activity, we have found significant type II fiber atrophy by muscle biopsy, at least some of which seems to be on the basis of defective lower motor neuron influence on muscle (with NCI). We are now attempting to analyse the mechanism in patients and trying to establish an animal model. This "distant effect" muscle weakness is often the most crippling aspect of cancer, and if the mechanism is known it might be treatable independent of treatment and response of the cancer itself.

Central Nervous System Disorders: Brain tumors -- Because viral-induced experimental brain tumor is a likely model for the etiology of at least some human brain tumors, the factors influencing the cell type of the experimental tumors, which can resemble most of the spontaneous human brain tumors, were elucidated and reported: inoculation of highly purified Schmidt-Ruppin Rous sarcoma virus into the subependymal cell plate of neonatal dogs produced only gliomas, while inoculation into the parietal subdural space or the cerebellar vermis produced sarcomas. Epilepsy -- The new syndrome of myoclonus epilepsy, limb-girdle dystrophy with "ragged-red muscle fibers," elevated blood lactate and pyruvate, and impaired oxidation of butyrate by muscle slices has been elucidated. The ultrastructurally and biochemically abnormal mitochondria of the "ragged red fibers" (a now-widely-used light-microscope-derived term introduced by us to describe fibers containing abnormal masses of bizzare mitochondria) may provide a more assessible way of learning about the metabolic defect of the brain causing the epilepsy. Spinocerebellar ataxias -- In two unrelated patients with Friedreich's ataxia, in whom we had previously shown impaired oxidation of pyruvate by their muscle slices, cultured skin fibroblasts have been shown to oxidize 1-$^{14}$C-pyruvate and 2-$^{14}$C-pyruvate at less than half the rate of (but U-$^{14}$C-glutamate at rates comparable to) those found in normal fibroblasts. Other degenerations -- Subacute sclerosing panencephalitis (SSPE) has come to be considered an effect of the rubeola virus -- a lingering infection with a "slow" form of the virus or an altered immune response to it, or both. We have reported SSPE existing in only one of identical twins, both of whom were studied clinically and immunologically in detail (with PRB, CF, NINDS). This evidence tends to downgrade certain suspected etiologic variables, e.g., host genetic composition, strain of rubeola virus, or general environmental factors but still compatible with others, e.g., prior or subsequent infections with other viruses or genetic composition other than that of the tested major histocompatability loci.

Methodology and Basic Cellular Mechanisms:

"Open-biopsy electromyography" (EMG) was reported as the first technique to give direct correlation of electrical activity of human muscle fibers during voluntary effort with the histochemistry of those fibers. It is done by taking the EMG apparatus into the operating room and recording at time of biopsy. With it, different physiologic firing patterns of type I and II neuron motor units were identified: type I units firing as a slow sustained rhythymic discharge during slight effort, and type II units firing as limited bursts of rapid discharges only during greater effort. With this technique it has further been reported that the pattern of short-duration small-amplitude overly-abundant potentials (SSAPs) on voluntary effort can sometimes come from regions showing no myopathy in the usual sense but instead consisting of type I fiber atrophy, type I fiber hypotrophy with or without central nuclei, and even "ordinary denervation" in myasthenia gravis patients. From this we have proposed that the former and widely used designation of "myopathic EMG" for the SSAP pattern is erroneous, since it could also be due to fractionation of the motor unit (a) on an "ordinary" neuropathic basis or (b) with type I fiber (or both-fiber) smallness on a proposed subtle neurogenic defect-of-trophic-factor basis. If this proves correct it alters the entire concept of the pathogenesis of a number of such diseases.

Tissue culture -- Cultured mouse embryo motor neurons have been used for seeking toxicity of ALS sera (v.s.) and for beginning histochemical, electron-microscopic, and scanning EM studies. We have reported on the first phases of biopsied normal and abnormal human muscle cultured with nerve supply. Muscle fibers become cytologically well differentiated but are not of two histochemical types, suggesting that type differences are controlled by the motor neurons. Ultrastructurally they resemble cultured animal muscle but have a poorly developed T-system and sarcoplasmic reticulum, perhaps explaining why they do not twitch in culture as do animal muscle fibers. No differences in general growth characteristics were evident in various kinds of neuromuscular diseases. To expand our opportunities, muscle biopsies from our patients with various neuromuscular diseases are being cultivated in collaboration with the Institute for Muscle Disease, New York, New York.

Electron-microscopy (EM) of cytochrome-oxidase-stained mitochondria appear to show that the intramitochondrial crystals of "ragged red fibers" lack that enzyme stainability. The new abnormality of "zipper tubules," identified histochemically, has been elucidated by EM.

The histochemical staining for phosphorylase in skeletal muscle was reported to be absolutely dependent on the amount of intrinsic glycogen in the tissue, which cannot be compensated for by excess glycogen to the medium.

A new nomenclature for human muscle fiber histochemical types has been submitted as a chairman-of-the-nomenclature-committee's draft to the other members of the Research Group for Neuromuscular Diseases of the World Federation of Neurology. The myofibrillar ATPase (pH 9.4) is the stain of choice for distinguishing two basic fiber types while other reactions, such as various oxidative enzymes, are useful for identifying various subtypes.

Biochemical studies -- With special methods, mammalian muscle sarcolemmal membranes have been highly purified and concentrated, as evidenced by marked enrichment for Na$^+$-K$^+$-stimulated-Mg$^{++}$ ATPase, acetylcholinesterase, and binding of $_\alpha$-bungarotoxin. Protein and glycoprotein bands from these fractions are now being analysed biochemically and histochemically. In addition, the skeletal muscle nicotinic cholinergic receptor molecule(s) is being purified and assayed by its binding with $^{125}$I-bungarotoxin and anti-$_\alpha$-bungarotoxin antibodies. This approach will provide new information on specific and non-specific membrane receptor and transport properties of normal muscle and, if it can be sufficiently minaturized, applied to normal and abnormal human muscle. Already we are studying location and distribution of receptor molecules of normal and abnormal human muscle with $^{125}I_\alpha$-bungarotoxin. Subcellular localization of a histochemical reaction we originally described, EDTA-acid-inhibited/activated ATPase, has been analysed by biochemical cell fraction and by electronmicroscopy of skeletal muscle. It is much greater in type I fibers (red, slow twitch) than in type II fibers (white, fast twitch) and lies especially in sarcoplasmic reticulum by both techniques, but to some extent in myofibrils by biochemical and histochemical evaluations.

Physiologic studies -- Modification of muscle histochemistry and contraction properties by altered functional needs was demonstrated: rat soleus doing more and different work because of denervation of its antagonist muscles increased its proportion of ATPase-dark (type II) fibers and its contractile speed (with LNNS, NINDS). A new method of more precisely evaluating elastic properties of active and resting skeletal muscle has been developed. It is based on the fact that the propagation rate of acoustical perturbations is a function of the elastic stiffness of the conducting material (muscle) (with NIAID). Special equipment has been designed and built, and a variety of isotropic materials tested. Preliminary results suggest that (a) previously used methods to evaluate elastic stiffness are inadequate and (b) active muscle is much stiffer than previously estimated.

Serial No. NDS(I)-62 MN/OC 915(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Histochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigator: W. King Engel, M.D.

Other Investigators:
Guy G. Cunningham
John R. Warmolts, M.D.
Alan D. Prince, M.D.
Husseiny I. Dessouky, M.D.
N. Bojji Reddy, M.D.
Peter K. Re', M.D.
Bernard M. Patten, M.D.
Michael L. Swerdlow, M.D.

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 2.7 |
| Professional: | 1.2 |
| Other: | 1.5 |

Project Description:

Objectives: (1) To study the cellular and subcellular localization of a variety of histochemical reactions in normal human skeletal muscle, neurons and peripheral nerves and to see how they are altered in neurologic disease. (2) To develop new histochemical techniques and to analyse mechanisms of the reactions. (3) To correlate histochemically defined types of muscle fibers with physiologic, ultrastructural, and biochemical properties. (4) To identify selective or non-selective involvement of one histochemical fiber type in the various neuromuscular diseases.

Methods Employed: Histochemical techniques for a number of reactions are being done on muscle and nerve tissue - they have been outlined in previous reports.

Patient Material: Biopsies obtained for diagnostic purposes were studied histochemically from Medical Neurology Branch patients as well as from consultation patients; patient biopsy material was also submitted by outside physicians and investigators. Some muscle biopsies were grown in tissue culture prior to histochemical study (see our Tissue Culture Project).

Major Findings: We reported that the histochemical staining for phosphorylase in skeletal muscle is absolutely dependent on the amount of intrinsic glycogen in the tissue, which cannot be compensated for by 100 fold glycogen excess added to the incubation medium. This is in contrast to the generally held belief, and means that phosphorylase can neither be localized nor quantitated accurately histochemically because it is absolutely dependent on the adjacence of the very labile metabolite glycogen. Demonstration of the presence of phosphorylase is acceptable but not of its absence, unless glycogen is known to be present (as it is in McArdle's disease = skeletal muscle phosphorylase deficiency). Likewise, phosphorylase cannot safely be used for muscle fiber typing histochemically. A new nomenclature for human muscle fiber histochemical types has been submitted as a chairman-of-the-nomenclature-committee's draft to the other members of the Research Group for Neuromuscular Diseases of the World Federation of Neurology. The myofibrillar ATPase (pH 9.4) is the stain of choice for distinguishing two basic fiber types while other reactions, such as various oxidative enzymes, are useful for identifying various subtypes.

Adaptive skeletal muscle responses, histochemical and physiological, to changes in environment or functional requirement have been identified in the rat (with LNNS, IR) (see our Functional Activities and Properties of Skeletal Muscle Project).

We have been asked to discuss the importance of histochemistry of muscle to the general pathologist at an international congress.

Metabolism of ketone bodies in skeletal and heart muscle has been compared histochemically. Numerous interrelated studies between this Project and other Medical Neurology Branch Projects were recorded under those other projects, for example:

a. New techniques of "Open-biopsy Electromyography" for direct correlation of electromyographic and histochemical data from the same fibers, in patients with various neuromuscular disorders - Electrophysiology Project.

b. Histochemical demonstration of revitalization of phosphorylase in regenerating muscle fibers of muscle phosphorylase deficiency disease (Myopathy and Tissue Culture Projects).

c. Histochemistry of human and animal muscle cells and neurons in tissue culture - Tissue Culture Project.

d. Histochemistry of motor neurons and peripheral nerves - ALS Project.

e. Histochemical demonstration of the characteristic lesions in

Duchenne dystrophy and the experimental models thereof - Myopathy Report.

f. Histochemical basis for suggesting that a number of erstwhile myopathic diseases may be neurogenic, e.g., myotonia congenita, paramyotonia congenita, myotonic atrophy (dystrophy), type I fiber hypotrophy with or without central nuclei, central core disease, and the type I fiber predominance form of the benign cogenital hypotonia syndrome - ALS Project.

g. Histochemistry was the basis of our new approach to defective motor neuron influences on muscle; the in toto vs. in portio analysis - ALS Project.

Significance to Bio-Medical Research and the Program of the Institute: It has been firmly established over the past eight years that histochemistry of muscle biopsies is the single most important laboratory evaluation of human neuromuscular disease, and that it is also extremely useful in (a) screening for correlating with electron microscopic studies, (b) correlation with electromyographic findings recorded from the same fibers and (c) evaluating animal models. This approach has provided insight into the possibility that certain diseases heretofore called "myopathies" may actually be caused by a form or neuronal abnormality, perhaps related to a defect of neuronal trophic function. This type of analysis (a) helps establish whether the basic pathogenesis and etiology are related to properties reflecting the metabolic "sameness" or the "differentness" of the lower motor neurons or muscle fibers, and (b) directs subsequent investigative studies toward analysing those two groups of properties of muscle and nerve cells. Obviously, knowing whether a certain neuromuscular disease is due to nerve cell or muscle cell abnormality is basic to understanding its cause and treatment. The phosphorylase reaction has been shown to be less useful for muscle fiber typing than formerly thought.

Proposed Course of Project: Further correlations of the results from light microscopy with those of electron microscopy, physiology and biochemistry will be made in human neuromuscular diseases and certain normal animal studies. Other animal models of human neuromuscular disorders will be produced and studied.

Honors and Awards: None

Publications:

Burke, R. E., Levine, D.N., Zajac, R.E., Tsairis, P., and Engel, W. K.: Direct correlation of physiological and histochemical characteristics in motor units of cat triceps surae. 4th Int. Cong. of Electromyography, Brussels, 1971. In press.

Martin, D. L. and Engel, W. K.: Dependency of histochemical phosphorylase staining on amount of cellular glycogen. J. Histochem. Cytochem. 20:476-479, 1972.

Campa, J. F. and Engel, W. K.: Histochemistry of motor neurons innervating slow and fast motor units in the cat. 4th Int. Cong. of Electromyography, Brussels, 1971. In press.

Engel, W. K. and Cunningham, G. G: Histochemistry in neuromuscular disease (NMD) pathology. 4th Congress of the European Society of Pathology, Budapest, 1973. In press.

Engel, W. K.: Current thoughts concerning the pathogenesis of various neuromuscular diseases. 3rd Symposium on Neuromuscular Disorders, Janske Lazne, Czechoslovakia, 1973. In press.

Serial No. NDS(I)-62 MN/OC 917(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

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|---|---|
| Principal Investigators: | W. King Engel, M.D.<br>Barry W. Festoff, M.D.<br>N. Bojji Reddy, M.D. |
| Other Investigators: | R. A. Pieter Kark, M.D.<br>John P. Blass, M.D.<br>Eugene C. Weinbach, M.D. |
| Cooperating Units: | U.C.L.A., School of Medicine, Los Angeles, California<br>Physiology and Biochemistry Section, LPD, NIAID |

Man Years:

| | |
|---|---|
| Total: | 2.4 |
| Professional: | 2.1 |
| Other: | 0.3 |

Project Description:

Objectives: (1) To seek and analyse biochemical abnormalities of neuromuscular and other human neurologic diseases; (2) to elaborate biochemical differences and similarities between "red" and "white" muscle of animals and between type I (similar to "red") and type II (similar to "white") individual muscle fibers of humans and animals; (3) to relate biochemical properties, and possible defects thereof, to specific subcellular components after their isolations and purification.

Methods Employed: Blood, spinal fluid, muscle biopsy, and cultured fibroblast samples of patients were studied with a variety of techniques:

a. Standard isotope techniques for oxidation of succinate, $\beta$-OH-butyrate, palmitate, and pyruvate have been adapted to tissue slices of small biopsy specimens of human muscle.

b. Standard oxygen electrode techniques for the oxidation by pure mitochondrial fractions, isolated from human muscle biopsies and cultured human skin fibroblasts, of succinate, $\beta$-OH-butyrate, palmitate, pyruvate, and NADH.

c. Adapted to mammalian muscle has been a technique of isolation and purification of rat sarcolemmal membrane by differential centrifugation combined with salt extraction of myofibrillar protein and subsequent rate-zonal density gradient separation, the membranes being obtained in the zone corresponding to 1.0-1.16M sucrose.

d. As an extension of "c" (above), the mammalian (rat) muscle nicotinic cholinergic receptor is being isolated.

e. $^{125}$I-$\alpha$-bungarotoxin is being used to identify the nicotinic cholinergic receptor population in biopsies of patients with various neuromuscular diseases.

f. Virtually fiber-type pure soleus (type I, red) and extensor digitorum longus (EDL) (type II, white) guinea pig muscle was fractionated into sarcoplasmic reticulum (SR) mitochondria, myofibrils, actomyosin, and myosin according to established procedures. These fractions were assayed at pH 9.4 (with calcium as the activating factor) for their ATPase activity without and with EDTA pretreatment at varying pHs (4-10.5) in media exactly corresponding to that used for histochemistry, in order to correlate biochemical subcellular localization with that of histochemistry.

g. Lactate, pyruvate, and creatine phosphokinase (CPK) of blood and spinal fluid.

h. Enzymes of glycogen metabolism in muscle -- phosphorylase, glycogen, amylo-1,6-glucosidase, phosphoglucomutase, and acid and neutral maltase.

Patient Material: Selected Medical Neurology Branch patients with neurological diseases, utilizing specimens obtained for diagnostic purposes.

Major Findings: a. In slices of human muscle biopsies we last year reported that muscle from more than half the patients with spinocerebellar degeneration and 1/3 of cases with idiopathic or familial neuropathy oxidized pyruvate at a rate $\geq$5 SE below the control mean. We have now demonstrated in the cultured skin fibroblasts of patients from two families with spinocerebellar degeneration (Friedreich's ataxia type) that same biochemical defect.

b. Muscle from 4 patients with myopathies characterized by ragged-red muscle fibers oxidized $\beta$-OH-butyrate $\geq$3 SE below the control mean.

c. In a family with hereditary myoclonus epilepsy, a concomitant myopathy (with ragged red fibers) with severe morphologic abnormalities of mitochondria and a biochemical defect of oxidation of $_\beta$-OH-butyrate by muscle slices was found. Assuming the same metabolic defect may exist in brain causing the epilepsy, skeletal muscle provides much more accessible tissue for its elucidation.

d. The sarcolemmal membrane fraction obtained is highly enriched for vesicular membranous fragments by electronmicroscopy, as well as for $Na^+$-$K^+$-stimulated-$Mg^{++}$-ATPase, acetylcholinesterase, and binding of $_\alpha$-bungarotoxin. The fragments also interact in appropriate concentrations with a number of cholinergic ligands. SDS-polyacrylamide electrophoresis of these membranes show several distinct and reproducible protein and glycoprotein bands. Biochemical and histochemical analysis of these bands is now being done.

e. Partial purification and solubilization (with the non-ionic detergent Lubol W-X) of the rat muscle nicotinic cholinergic receptor has been accomplished, its assay being its interaction with $^{125}$I-$_\alpha$bungarotoxin prepared in our laboratory. A specific, high affinity, anti-$_\alpha$-bungarotoxin antibody has also been prepared, in both goat and rabbit, with the intent of using it for quantitating amounts of toxin and for ultrastructural localization of the cholinergic receptor on membrane fragments and tissue slides by the new unlabelled antibody method.

f. In initial studies we have successfully developed a technique of applying the $^{125}$I-$_\alpha$-bungarotoxin to thin fresh-frozen sections of human skeletal muscle and after autoradiographic development applied enzymatic counterstains, e.g., for acetylcholinesterase. These have shown specificity of the bind to the motor end plate region. Studies are in progress to seek alterations thereof in various neuromuscular diseases.

g. Biochemical observations of ATPase activity following acidic EDTA pre-incubation (pH 4.5) was as follows: the specific ATPase contributed by each fraction of the type-I-fiber muscle was comparatively and considerably greater than that of the type-II-fiber muscle, indicating that they all contribute to the greater histochemical staining of type I fibers.

h. Analysis of various enzymes of glycogen metabolism in muscle biopsies of our patients has resulted in diagnoses such as phosphorylase deficiency and acid maltase deficiency.

Significance to Bio-Medical Research and the Program of the Institute: Elucidation of biochemical abnormalities, particularly in the realm of enzymes and other proteins, is important to the understanding of neurologic and neuromuscular diseases, and in seeking means of therapy. Putting the various abnormalities in proper perspective regarding pathogenesis and disease specificity is often neglected, but it is vital to planning future investigative and therapeutic procedures. Having comparative studies of biochemical differences between type I and type II skeletal muscle fibers will help provide a basis for understanding the pathogenesis of the many neuromuscular disorders preferentially affecting one of these fiber types, as detailed in the Histochemistry Project. The precise localization and characterization of normal biochemical properties to each type of subcellular element of the muscle fiber can serve as the basis for seeking biochemical defects thereof in various human neuromuscular diseases.

The spinocerebellar ataxias, usually hereditary, have long been resistant to yielding up their secrets to scientific analysis, but a beginning is now being made in their biochemical analysis. (We last year reported one patient with intermittent cerebellar ataxia who was found to have pyruvate decarboxylase deficiency.)

Proposed Course of Project: The above techniques will be further perfected and applied to muscle from a variety of human neuromuscular and central nervous system disorders to seek further details of biochemical defects therein, as well as the biochemical differences and similarities of normal human type I and type II fibers, insofar as personnel and space permit. (Progress would be enhanced by obtaining one full-time technician for the project, none currently being available.)

Honors and Awards: None

Publications:

Kark, R. A. P., Blass, J. P., and Engel, W. K.: Oxidative metabolism in small samples of normal and diseased human muscle. II Intern. Cong. on Muscle Diseases, Nov. 22-26, 1971, Perth, Australia. In press.

Tsairis, P., Engel, W. K., and Kark, R. A. P.: Familial myoclonic epilepsy syndrome associated with skeletal muscle mitochondrial abnormalities. Neurology 23: 408, 1973.

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1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Myopathies

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Jerry R. Mendell, M.D.
John R. Warmolts, M.D.

Other Investigators: John N. Whitaker, M.D.
Carolyn Derrer
Alan Borenstein, M.D.
Dennis L. Murphy, M.D.
Richard W. Price, M.D.
Barry E. Levin, M.D.

Cooperating Units: Laboratory of Clinical Science, NIMH

Man Years:

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|---|---|
| Total: | 3.2 |
| Professional: | 2.2 |
| Other: | 1.0 |

Project Description:

Objectives: To more fully elaborate the clinical, histochemical, biochemical, and ultrastructural abnormalities of patients with the various myopathies. To further sub-classify patients in each category using those parameters. To treat myopathic disorders by different methods in order to learn which is most effective within each disease category. To produce animal models of pathogenic phenomena.

Methods Employed: Most of the details of techniques applied to human material are described in the various methodological projects of this Branch. Therapeutic trials were conducted with very careful clinical and laboratory examinations to document change of disease state. For comparison with Duchenne muscular dystrophy, non-structural vascular lesions of muscle were produced in animals, the rise of serum "muscle enzymes" followed, and preventative drugs sought.

Patient Material: Diagnostic material from Medical Neurology Branch patients and outside patients from whom diagnostic muscle biopsies were obtained and sent here for study.

Major Findings: We have previously proposed a new hypothesis, based on muscle histochemical studies, concerning the pathogenesis of Duchenne's progressive pseudohypertrophic muscular dystrophy (DMD). Because the earliest histochemical change is a grouped pattern of degenerating and regenerating muscle fibers, it was hypothesized that the disease is caused by an abnormality of small arterial blood vessels on either a structural or functional basis. That principle was established and the hypothesis shown to be possible by the production of identical early and late muscle lesions after single and repeated embolization of rabbit arteries (structural arterial occulsion). We then reported production of the same early and late Duchenne-like muscle lesions with non-structural, presumably functional, lesions of intramuscular blood vessels by combining a subthreshold vascular factor (aortic ligation) and a subthreshold dose of a vasoactive amine (either serotonin or norepinephrine), thereby establishing the possibility of a functional abnormality of blood vessels and/or vasoactive substances affecting them being the basis of Duchenne dystrophy (thus like small "strokes" or "transient-ischemic-attacks" of muscle). We have now reported that in this model, following each dose of serotonin or norepinephrine in the previously ligated animal there is a striking rise of "muscle enzymes" (CPK, SGOT, SGPT, LDH) in the serum (peak about 12 hours), which returns to normal by 3-5 days. Repeated doses of serotonin cause repeated rises of those enzymes. This demonstrates a further parallel of the model with human DMD and also provides an index against which various therapeutic drugs can be evaluated. So far one has been shown to nearly completely prevent the serotonin-induced rise of muscle enzymes.

In the patients themselves with Duchenne muscular dystrophy, we have now reported a defective uptake of serotonin by platelets (with NIMH) -- a similar defect, of uptake of vasoactive amine, if present in the region of intramuscular small arterial vessels, could result in prolonged action and consequent functional ischemia.

Contrary to a few studies by others, we have reported that there is no abnormality of various catecholamines and indolamines in CSF or urine in DMD patients (with NIMH).

To study the role of blood vessel innervation in myopathies of possible vascular origin, e.g., Duchenne muscular dystrophy, as well as in cerebro-vascular disease, we are making antibodies to dopamine-$_{\beta}$-hydroxylase.

In myotonic atrophy (myotonic dystrophy), several lines of evidence continue to suggest to us that muscle involvement in this disease is not myopathic, but due mainly to an altered nerve influence on muscle (and therefore should not be listed in the dystrophy category).

In myotonia congenita and paramyotonia congenita, also thought by others to be myopathies, we have now postulated a major neurogenic aspect to the pathogenesis, on the basis of histochemical evidence. Other erstwhile "myopathies" now suspected of being mainly or partly neurogenic are detailed in our Amyotrophic Lateral Sclerosis Project.

Amyloid myopathy, with sporadic and familial forms, has been discovered and one, periodic case, in whom a monoclonal gammopathy was found, is undergoing treatment with melforan.

In progressive external ophthalmoplegia (sometimes called "ocular myopathy") we have previously reported that "ragged-red fibers" are present in clinically normal limb muscle (as well as weak muscle) and are characteristic of the syndrome. That fiber-descriptive term is now being widely used. Such fibers have excessive lipid droplets and excessive and enlarged mitochondria with bizarrely proliferated membranes. We also reported last year that muscle slices from 4 of 5 patients with myopathies characterized by "ragged-red" muscle fibers had diminished oxidation of 3-$^{14}$C-D,L-$_{\beta}$-hydroxybutyrate, i.e., 3.5 SE below the mean for 19 normal or disease-control patients (4 normals, 4 with non-neuromuscular diseases, 11 with various myopathies). In two cases, 2-$^{14}$C-pyruvate was also oxidized at somewhat low rates but 1,4-$^{14}$C-succinate oxidation was normal in all. We now have found the same ragged red fibers with the same ultrastructural and biochemical abnormalities in a family.

Myopathy with emphasis on mitochondrial abnormalities was identified in childhood onset myoclonus epilepsy, persistent lactic-acidosis, and a limb-girdle myopathy (see our Electron Microscopy and Biochemistry Projects). The mitochondria were bizzare, with whorled cristae and crystalline inclusions. These patients did not have ophthalmoplegia.

In skeletal muscle phosphorylase deficiency (McArdle's disease) hitherto thought to be a complete genetic absence of the muscle enzyme phosphorylase, we have demonstrated presence of that enzyme in the muscle fibers from biopsies of such patients grown (regenerating) in tissue culture, as well as in regenerating fibers *in vivo*. This "revivability" of the enzyme offers a new therapeutic possibility for the patients (see our Tissue Culture Project).

The polymyositis/dermatomyositis disease complex, when beginning over the age of 40, especially in males, is commonly associated with, and perhaps due to, a small distant cancer. The childhood form is not associated with cancer, but both age groups might have similar pathogenic mechanisms. The method of treatment we introduced to this disease 5 1/2 years ago, high-single-dose alternate-day glucocorticoid (prednisone), has now proven in our hands to be the best available both for children and adults without or with cancer. It has greater therapeutic benefit, fewer side-effects, and is easier to manage. Our experience now includes about 28 carefully studied adults and children, many of them considered therapeutic failures on other regimens.

Last year we reported a new hypothesis on a mechanism of muscle damage in some cases of the polymyositis/dermatomyositis (PM/DM) complex. On the basis of our reported finding of deposits of immunoglobulin G, immunoglobulin M, and $\beta_{1c}$ globulin (C3), alone or incombination, in skeletal muscle blood vessels of 15 of 36 cases of PM/DM (a finding now confirmed by others), especially in childhood dermatomyositis, we suggested that one of the mechanisms leading to muscle damage in some patients with inflammatory myopathy may be vascular abnormality mediated through deposits of immunoglobulin and C3, possibly in the form of immune complexes. This supports our earlier hypothesis that some cases of PM/DM may be another form of blood-vessel abnormality of muscle. The immunological abnormality could in turn be caused by a viral infection and/or an associated cancer, or the cancer and the "immuno-myopathy" could both be viral-induced. Consequently, a search for Australian antigen has been now made, but none is detectable, either circulating or bound to blood vessels or muscle cells, in any of our PM/DM patients (with CC). Viral isolation studies involving short and long-term cultures of muscle from polymyositis and dermatomyositis patients have so far failed to reveal a transmissible agent (with NCI and PRB, NINDS).

To study the direct effect of virus on muscle we are developing an animal model of viral myositis (see our Immunology Project).

In one case of late-onset rod disease an associated unusual serum paraprotein, IgG/$\lambda$ myeloma protein, has been found; its possible relation to the muscle degeneration is being explored. Intranuclear rods, identical histochemically and ultrastructurally to those of the cytoplasm, were also found in him, suggesting an origin from a contractile or other protein common to both nuclear and cytoplasmic sites. (See our Immunology and Electron Microscopy Projects.)

A new clinically delineated "levitated arms syndrome" has been described in 2 cases and the cause identified as iatrogenic intramuscular injections into the deltoids; one was successfully treated by surgical transection of fibrous bands.

A bizzare polysaccharide material has been found as the storage material in muscle in a rare type of cardio-skeletal myopathy

To more precisely analyse the disease alterations of subcellular components of muscle fibers, we are developing methods of isolating for biochemical study the various fractions, including a muscle membrane fraction (see our Biochemical Project).

The principal investigator organized the scientific program of a 3-day symposium on neuromuscular diseases in Houston, November 1972, under the sponsorship of the Veterans Administration and Baylor Medical School.

Significance to Bio-Medical Research and the Program of the Institute: The myopathies represent a group of disorders rather similar clinically, the detailed differences of which have only begun to be worked out in the recent few years. One of the major methods for distinguishing these disorders is by histochemistry of the muscle biopsy. Other methods include electron microscopy, biochemistry, and immunology. Hence, the distinguishing of various new forms of disease represent a step toward the overall understanding of this group of disorders. It is only when the detailed differences of the various forms of myopathy are analysed that the pathogenesis, etiology, and treatment can be understood. In fact, we are now proposing that some erstwhile "myopathies" (such as "myotonic dystrophy") are the result of abnormal neural influence on muscle. Identification of characteristic histochemical and biochemical defects (such as the inability to utilize long-chain fatty acids and phosphorylase deficiency) in the myopathies provides leads to further explore the pathogenesis and possible treatment.

Specific or semi-specific structural changes of certain elements, such as mitochondria, especially when coupled with a metabolic defect, such as lactic-acidosis, provide leads toward finding a specific metabolic defect.

The new histologically based hypothesis of ischemia as the cause of Duchenne muscular dystrophy raises for consideration the possible use of drugs to increase the blood flow through perfusion vessels of muscle.

The beneficial response obtained in the therapy of dermatomyositis/polymyositis demonstrates an additional regimen available in the management of such disorders.

Finding that immunoglobulin complexes in intramuscular blood vessels may be a factor in the muscle damage offers a new avenue for possible therapy.

Finding that muscle fibers in "myophosphorylase deficiency" are capable of producing phosphorylase under conditions of regeneration not only demonstrates a whole new concept of the disease that is needed to replace the "absence of a gene" idea, but that "revivable" phosphorylase means a new possibility of "treating the enzyme" and thus the patient.

Identification of the iatrogenic cause of the "arm levitation syndrome" can result in its prevention, and demonstration of a treatment can be of benefit to the patients so afflicted.

Proposed Course of Project: The studies underway are part of a long-term project which will continue for several years.

Honors and Awards: None

Publications:

Engel, W. K.: Duchenne muscular dystrophy: A histologically-based ischemia hypothesis and comparison with experimental ischemia myopathy. Proc. 59th Ann. Mt. Intern. Acad. Path. Williams & Wilkins Co., Baltimore, Md. In press.

Engel, W. K.: "Ragged-red fibers" in ophthalmoplegia syndromes and their differential diagnosis. II Intern. Cong. on Muscle Diseases, Nov. 22-26, 1971, Perth, Western Australia. In press.

Mendell, J. R., Engel, W. K. and Derrer, E. C.: Plasma enzyme elevations in rats with functional ischemia -- A possible model of Duchenne muscular dystrophy. Nature 239: 522-524, 1972.

Mendell, J. R., Murphy, D. L., Engel, W. K., Chase, T. N. and Gordon, E.: Catecholamines and indolamines in patients with Duchenne muscular dystrophy. Arch. Neurol. 27: 518-520, 1972.

Murphy, D. L., Mendell, J. R. and Engel, W. K.: Serotonin and platelet function in patients with Duchenne muscular dystrophy. Arch. Neurol. 28: 239-242, 1973.

Engel, W. K., Borenstein, A., DeVivo, D. C., Schwartzman, R. J., and Warmolts, J. R.: High-single-dose alternate-day prednisone (HSDAD-PRED) in the treatment of the dermatomyositis/polymyositis complex. Trans. Amer. Neurol. Assn. 97: 272-275, 1972.

Levin, B. E. and Engel, W. K.: Arm levitation syndrome from iatrogenic muscle fibrosis. Neurology 1973. In press.

Engel, W. K. and Borenstein, A.: Histochemical evidence raising the question of a neurogenic aspect in myotonia congenita (MC) and paramyotonia congenita (PM). Neuropathology Meeting, 1973, Freeport, Grand Bahama. In press.

Serial No. NDS(I)-63 MN/OC 1037(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Tissue Culture Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Mark S. Horwich, M.D.
Robert I. Roelofs, M.D.

Other Investigators: Valerie Askanas, M.D.
Jerry R. Mendell, M.D.
Priscilla B. Chauvin

Cooperating Unit: Tissue Culture Laboratory, Institute for Muscle Diseases, New York, N.Y.

Man Years

| | |
|---|---|
| Total: | 2.4 |
| Professional: | 1.4 |
| Other: | 1.0 |

Project Description:

Objectives: To study the growth characteristics, histochemical reactivities, immunologic properties, and electron microscopic details of animal and human skeletal muscle and motor neurons grown in tissue culture. To compare the growth and histochemical characteristics of "red" vs. "white" muscle in culture. To study interactions between lower motor neurons and muscle fibers in culture. To seek growth-inhibitory ("toxic") factors in body fluids of our patients, using cultured neurons or muscle cells as test objects. The tissue is from either normal chick, mouse or rat embryo or is normal or abnormal biopsied adult or child human tissue, especially muscle.

Methods Employed: Standard tissue culture techniques are used as a basis, modified as necessary to obtain good growth from human cells. Applied to the cultured cells are other techniques being used in the Branch, e.g., histochemistry, electron microscopy, and immunology.

Patient Material: A portion of the open muscle biopsy from certain adult and child patients with neuromuscular disease is available for culture purposes. Needle biopsies, used for repeat specimens, of such human muscle can also be grown successfully.

Major Findings: Although tissue culture techniques have been used extensively in studying the development of muscle from animal embryos, human skeletal muscle is difficult to culture. We have developed and published a system in which we achieve reproducible growth for 80% of explants of muscle biopsies of children and adults. It was reported that the morphologic and histochemical (myofibrillar ATPase and DPNH tetrazolium reductase) development of normal and pathologic (neuropathies, inflammatory myopathy, central core disease, myophosphorylase deficiency and muscle with abnormal mitochondria) human muscle is similar to that of chick embryo muscle, but is delayed, slower, and has a greater propensity to form fibers away from the explant. No abnormal growth patterns.

Electron microscopic studies of normal cultured human muscle disclosed subcellular features like those of chick embryo muscle in culture, except the human fibers have a relatively poor development of the T-tubules and sarcoplasmic return. Perhaps these defects correlate that the cultured human fibers do not contract spontaneously. The new human muscle fibers in culture appear to be derived from myoblasts, apparently derived from satellite cells of the explanted muscle, which fuse to form multinucleated cells and differentiate into mature fibers.

Serum from 70% of amyotrophic lateral sclerosis (ALS) patients has been reported by others to be toxic to three-day-old mouse spinal cord neurons maintained in tissue culture. We tried to confirm those results, using both three-day-old and embryonic mouse cords, and so far have failed to find any difference between 7 ALS sera and 19 control sera using more than 200 cultures.

In skeletal muscle phosphorylase deficiency (MPD) (McArdle's disease), hitherto thought to be a complete absence of the phosphorylase genome in skeletal muscle, we have reported the presence of that enzyme in muscle fibers from biopsies of such patients grown (regenerating) in tissue culture, as well as in fibers regenerating in vivo. Thus we tentatively conclude that in MPD genetic coding for a form of phosphorylase activity is present in the skeletal muscle fibers regenerating in vitro and in vivo. Several hypotheses can be considered to explain the subsequent absence of phosphorylase activity in mature fibers in vivo. (a) With muscle fiber maturation there may be loss of a specific enzyme which normallly maintains phosphorylase production, survival or activity. (b) A specific protease may develop in the mature fibers which selectively inactivates myophosphorylase. (c) A normally repressed "myophosphorylase repressor gene" may be derepressed, inhibiting the gene for myophosphorylase production. (d) A normally inactive or absent myophosphorylase inhibiting or destroying enzyme (or other factor) may become active, impairing myophosphorylase enzyme survival or activity. The rejuvenation of phosphorylase in muscle fibers probably does not involve the complex cascade of mechanisms that activate phosphorylase b to a, such as the kinase, kinase-kinase and cyclase systems, since in the hisotochemical method one uses ordinary AMP which makes active directly the 180,000 molecular weight phosphorylase b dimer and does require the b to be tetramerized to the a form to gain activity. Whatever the explanation in MPD, these studies raise the therapeutic possibility of finding a way to continuously rejuvenate the enzyme in mature fibers, just as occurs spontaneously in the regenerating muscle fibers.

Histochemistry of the cultured normal and abnormal human muscle and animal neurons is in progress; electromicroscopic histochemistry and scanning electromicroscopy are in the early beginning stages.

Significance to Bio-Medical Research and the Program of the Institute: In addition to determining the histochemical properties of normal muscle and its growth characteristics *in vitro*, the studies of pathologic human muscle *in vitro* will be used to seek disturbed growth patterns, and morphologic and biochemical abnormalities. More particularly, ways of improving deficient growth will be sought. Agents found to improve deficient growth *in vitro* will then be tested for their usefulness in the patient with muscle disease to promote growth of abnormal muscle or to retard its degeneration. Study of motor neurons grown *in vitro* is part of a multilateral approach to diseases which preferentially involve human motor neurons, such as amyotropic lateral sclerosis. Finding that phosphorylase activity can be revitalized in regenerating muscle fibers phosphorylase deficiency muscle opens a new therapeutic possibility in this disease. Failure to confirm a toxic factor in ALS sera means that other possibilities for pathogensis must now be considered.

Proposed Course of Project: Further studies of normal and pathologic human muscle *in vitro* will be expanded.

Honors and Awards: None

Publications: Roelofs, R. I., Engel, W. K., and Chauvin, P. B.: Histochemical demonstration of phosphorylase activity in regenerating skeletal muscle fibers from myophosphorylase deficiency patients. *Science*. 177:795-797, 1972.

Engel, W. K., Relofs, R. I., Re', P. K. and Martin, D. L. Histochemically demonstrable phosphorylase in regenerating skeletal muscle fibers of myophosphorylase deficiency. (McArdle's disease). *Trans. Amer. Neurol. Assn*. 97:175-178 1972.

Mendell, J. R., Roelofs, R. I., and Engel, W. K.: The ulterstructural development of explanted human muscle in tissue culture. *J. Neuropathol. Exp. Neurol*. 31:433-446, 1972.

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1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Amyotrophic Lateral Sclerosis (ALS) and Other Lower Motor Neuron Diseases, Including Peripheral Neuropathies and Spinocerebellar Ataxias

Previous Serial Number: Same

| | |
|---|---|
| Principal Investigators: | W. King Engel, M.D.<br>John R. Warmolts, M.D.<br>Bernard M. Patten, M.D.<br>Mark S. Horwich, M.D. |
| Other Investigators: | John N. Whitaker, M.D.<br>Peter K. Re', M.D.<br>Thomas N. Chase, M.D.<br>Justino F. Campa, M.D.<br>Robert J. Schwartzman, M.D.<br>R. A. Pieter Kark, M.D.<br>John P. Blass, M.D.<br>Alan Rapoport, M.D.<br>Barry W. Festoff, M.D.<br>Alan D. Prince, M.D.<br>Stephen B. Guss, M.D.<br>Randall J. Lewis, M.D.<br>Michael L. Swerdlow, M.D. |
| Cooperating Units: | Laboratory of Clinical Science, NIMH<br>Dermatology Branch, NCI<br>Surgery Branch, NCI<br>Clinical Research, NEI<br>Depts. of Neurology & Biochemistry, U.C.L.A., School of Medicine, Los Angeles<br>Dept. of Neurology, University of Miami, School of Medicine, Miami |

Man Years:

| | |
|---|---|
| Total: | 2.4 |
| Professional: | 2.2 |
| Other: | 0.2 |

Project Description:

Objectives: In ALS and other diseases affecting the lower motor neurons, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, subforms; (c) specific or symptomatic therapeutic response; (d) new methods of analysing the abnormalities; and (e) animal models of the human pathophysiologic states.

Methods Employed: The techniques of histochemistry, biochemistry, electron microscopy, immunology, tissue culture, electromyography and autoradiography were as detailed in other project reports on those methods. Additional methods were as follows. Double-blind, single-blind and non-blinded therapeutic trials, the efficacy of which is judged by clinical testing, functional evaluation, and serial quantitative evaluation of muscle function using an apparatus designed by us for quantitating isometric muscle tension. Analysis of motor areas of brain and spinal cord by histochemistry and electron microscopy. Biochemical assays of several parameters of lipid metabolism (with U.C.L.A.). Animal studies to evaluate recoverability of sensory function in the monkey following ablation of the primary somatosensory projection cortex (with NIMH). Animals implanted with experimental cancers to study the distant effect of cancer on the neuromuscular system.

Patient Material: Medical Neurology Branch patients and Cancer Surgery patients, who had diagnostic muscle biopsy and other diagnostic procedures.

Major Findings:

## MOTOR NEURON DISORDERS

In ALS, different approaches continue to fail to disclose the cause. Another investigator has reported that sera from 70% of ALS patients but none (sic!) of numerous disease-controls was selectively toxic to motor neurons of 3-day mouse embryos in tissue culture. However, in a blinded study of about 200 cultures we fail to find any difference between normal and ALS sera -- neither was especially toxic to the cultured neurons. We are continuing to seek the explanation of differences between our study and the other one.

In hyperparathyroidism we have shown that there is neurologic abnormality in nearly every case, the most common involvement being of lower motor neurons, sometimes with bulbar involvement, tongue tremors, and brisk reflexes giving an ALS-like syndrome, which reverts to or toward normal following surgical treatment of the hyperparathyroidism (with NIAMD). The only CSF abnormality detected was a low phosphate in about half the cases.

Benign focal amyotrophy has been described as a disorder simulating the lower motor neuron aspects of ALS at its onset but of spontaneously limited progression.

We reported that of 18 histochemical reactions done on the cat lumbar cord in an attempt to learn metabolic particularities of $_\alpha$-motoneurons in the anterior horn of the spinal cord (the ones preferentially involved in ALS), only two distinguished them. $_\alpha$-motoneurons of both fast-twitch and slow-twitch motor units were rich in phosphorylase and poor in succinate dehydrogenase, in contrast to the opposite characteristics, of $_\gamma$-motoneurons, Renshaw cells, and other interneurons. Thus one theoretical possibility is that ALS might be related to an abnormality of glycogen metabolism.

New hypotheses on the pathogenesis of some neuromuscular disorders that we reported previously have continued to be our "first choice" explanation for those diseases -- specifically that some erstwhile "myopathic" disorders are actually caused by a lower motor neuron abnormality. It is proposed that in spite of a "myopathic" (which we consider often to be a misnomer) EMG, certain disorders are not myopathic but rather neurogenic. (A new approach to EMG studies is described in the Electromyography and Clinical Neurophysiology Project.) As part of this formulation, details have been proposed regarding how a motor neuron might be defective in a variety of qualitative or quantitative ways with a variety of time courses toward death. Each of these could give a different clinical disorder. Diseases newly considered to be probably neurogenic are type II muscle fiber atrophy (cachectic and disuse atrophy, corticosteroid atrophy ["myopathy"]), myasthenia gravis, facilitating myasthenic syndrome, myotonic atrophy (myotonic "dystrophy"), myotonia congenita and paramyotonia congenita, type I fiber hypotrophy with central nuclei, central core disease, benign congenital hypotonia (with type I fiber predominance), and facioscapulohumeral syndrome (some cases). Possibly neurogenic (perhaps only in a small part) are thyrotoxic atrophy ("myopathy"), some cases of facioscapulohumeral disease, focal loss of cross-striations, and late-onset and congenital rod disease. From such proposals a new classification of neuromuscular disorders has been reported. A new clinico-pathologic syndrome of type I muscle fiber smallness without central nuclei has been found in a child with progressive weakness; it was proposed to be of neural origin.

Further evidence was adduced by ourselves and others to support our earlier hypothesis that central core disease is more likely to be an abnormality of the neural influence on muscle than a myopathic disorder as originally proposed by others.

On the basis of histochemistry and electron microscopy, three new disorders were described, (a) a new form of central core disease in which there is a "hot spot" of enzymatic activity and lipid droplets in the center of the core, (b) focal loss of cross-striations, possibly due to defective motor neuron influence on muscle, and (c) type I fiber smallness without central nuclei. A new form of the benign congenital hypotonia syndrome was described, consisting of marked type I muscle fiber predominance -- this was proposed to be caused by an in utero time-limited neuropathy affecting mainly the type I motor neurons.

The generalized muscle cachectic atrophy and weakness, which is a common and often the most crippling manifestation of early focal (or metastic) cancer as well as of hypercorticosteroidism (Cushing's syndrome and iatrogenic) and disuse, histochemically is selective atrophy of type II muscle fibers. We have previously reported a new hypothesis to the effect that this may be a pan-neuropathy of all lower motor neurons to which the type II muscle fibers are more susceptible, not a myopathy as is usually stated. That proposal was supported by our report that the ultrastructural aspects of type II atrophy are qualitatively indistinguishable from typical denervation atrophy, but milder in degree. We have shown that there is no alteration of the EMG pattern in type II atrophy, either early or when the patient is severely wasted and weak, probably because the early-firing motor units usually measured during voluntary effort are the type I units (see our Electromyography and Clinical Neurophysiology Project). We have found (with NCI) in patients with non-neurologic, localized (non-metastatic) cancer, before there is clinical evidence of anorexia, weight loss, or decreased daily activity, significant type II fiber atrophy by muscle biopsy. The mechanism of the atrophy in such early-cancer patients is unknown, but at least some of it seems to be on the basis of defective lower motor neuron influence on muscle. We are now trying to produce an animal model of the cancer-induced type II atrophy (with NCI). We also plan to turn our attention to ways to prevent or treat type II atrophy, which would significantly benefit cancer patients even if the basic neoplasm could not be excised.

We have begun using autoradiography with $\alpha$-bungarotoxin to identify post-junctional receptor substance in human muscle fibers and to seek alterations of its spacial pattern in various neuromuscular diseases, to learn more about possible alterations of lower motor neuron influence on muscle.

## PERIPHERAL NEUROPATHIES

Our treatment of some cases of idiopathic peripheral neuropathy with high-single-dose alternate-day prednisone (HSDAD-Pred) continues to be very successful and necessary, e.g., for as long as 8 years in an adult and 5 1/2 years in a child. Correlative studies indicate that the patients most likely to respond are dysschwannian in type (slow nerve conduction times), relapsing, with elevated CSF protein, but even some patients with a dysneuronal type of peripheral neuropathy, non-relapsing, or normal CSF have responded (see our Myopathy Project).

Immunoglobulin survey of a large group of cases of peripheral neuropathy (PN) currently considered idiopathic disclosed elevation of serum IgM in some, suggesting an immunologic pathogenesis in them. A new disorder was discovered in seven patients, family sensory PN with elevated serum IgA, shown to be due to IgA overproduction in the small bowel tissue -- on this basis a new hypothesis was introduced, one of possible familial susceptibility to infection in the gut tissue (in which there are excessive eosinophils) that causes through, or parallel to, the IgA overproduction a metabolic abnormality affecting sensory nerves.

A dysneuronal neuropathy secondary to dapsone therapy was identified, with marked clinical improvement following discontinuation of the drug (with NCI). The peripheral neuropathy of xeroderma pigmentosa was identified and its possible etiology speculated upon: it was hypothesized that the previously known defective DNA-repair mechanism might cause a shortened life in non-mitotic (neuronal) cells (enhanced neurologic "aging") because of inability to properly repair ordinarily banal neuronal DNA injuries (with NCI). Acquired pupillary dysautonomia (light-hyposensitive, tonic, mecolyl-hypersensitive) has been identified and shown to be a usually-overlooked but not infrequent (11 of 16 patients) accompaniment of idiopathic non-familial neuropathy, especially when there is a major sensory component (with NEI).

## SPINOCEREBELLAR ATAXIAS

In only a very few of the various types of herediatry spinocerebellar degeneration is a metabolic abnormality known. Previously we have reported pyruvate decarboxylase deficiency in a patient with intermittant cerebellar ataxia (who also had excessive lipid droplets in his skeletal muscle fibers). We also have reported previously, using a system we developed for applying radioisotopic biochemical assays to small samples of biopsied human muscle, that the muscle biopsy slices from more than half the patients with spinocerebellar degeneration (as well as 1/3 of cases with idiopathic or familial neuropathy) oxidized pyruvate at a rate $\geq 6$ SE below the control mean; the oxidation rates were not related to the severity of any neuropathic changes in the muscle. Most recently, in two unrelated patients with Friedreich's ataxia, in whom we had previously shown impaired oxidation of pyruvate by their muscle slices, cultured skin fibroblasts have been shown to oxidase 1-$^{14}$C-pyruvate and 2-$^{14}$C-pyruvate at less than half the rate of (but U-$^{14}$C-glutamate at rates comparable to) those found in normal fibroblasts (see our Biochemistry Project).

Significance to Bio-Medical Research and the Program of the Institute: New details have been accumulated on the morphologic and biochemical substrata of at least some patients with yet-idiopathic forms of ALS, peripheral neuropathies, the spinocerebellar ataxias, and other diseases of the lower motor neuron. Identifying known and treatable underlying diseases (e.g., hyperparathyrodism, dapsone toxicity) in some of these clinical syndromes provides such patients with cures and gives possible leads to understanding the more numerous yet-idiopathic cases. New aspects of the beneficial use of prednisone in chronic neuropathy indicate additional patients should be tried on the drug on a prolonged program, every-other-day, even if they have been severely paralysed months or longer.

If our proposals are correct that some erstwhile myopathic disorders are actually caused by abnormalities of the lower motor neuron, then all searches for the basic abnormality that are focused on the muscle represent explorations in the wrong forest -- we must direct our attention to the motor neuron rather than the muscle.

Fuller understanding of the mechanism by which a focal cancer produces generalized muscle weakness and wasting could lead to a means of treating that often-most-disabling aspect of cancer even when the basic neoplasm itself cannot be treated -- such would be of major benefit to a great number of cancer patients.

Proposed Course of Project: To more fully develop studies underway in the hope that identification of metabolic, immune, or infectious etiologies will lead to a means of treating and preventing these disorders. To further explore the interactions of motor neuron and muscle, and disorders thereof. To undertake additional therapeutic trials. We are intrigued with the possibility of treating the cachectic muscle wasting aspects of cancer patients.

Honors and Awards: None

Publications:

Campa, J. F. and Engel, W. K.: Histochemistry of motor neurons innervating slow and fast motor units. In Desmedt, J. E., (ed.): New Developments in Electromyography and Clinical Neurophysiology. Basel, Karger, 1972. In press.

Engel, W. K.: Motor neuron disorders. In Shy, G. M., Goldensohn, E.S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease. In press.

Engel, W. K.: Central core disease and focal loss of cross striations. In Shy, G. M., Goldensohn, E. S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease. In press.

Engel, W. K.: Rod (nemaline) disease. In Shy, G. M., Goldensohn, E. S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease. In press.

Engel, W. K.: Muscle fiber hypotrophies. In Shy, G. M., Goldensohn, E. S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease. In press.

Engel, W. K.: Basic aspects of muscle abnormalities. In Shy, G. M., Goldensohn, E. S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease. In press.

Engel, W. K.: Basic aspects of lower motor neuron abnormalities. In Shy, G. M., Goldensohn, E. S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease. In press.

Rapoport, A. M. and Guss, S. B.: Dapsone induced peripheral neuropathy. Arch. Neurol. 27: 184-185, 1972.

Engel, W. K. and Warmolts, J. R.: The motor unit: Diseases affecting it in toto or in portio. In Desmedt, J. E., (ed.): New Developments in Electromyography and Clinical Neurophysiology. Basel, Karger, 1972. In press.

Engel, W. K. and Warmolts, J. R.: Multiplicity of muscle changes postulated from motoneuron abnormalities. In Cassens, M. (ed.): Muscle Biopsy. New York, Marcel Dekker, Inc., 1972, pp. 229-253.

Serial No. NDS(I)-65 MN/OC 1189(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Episodic Weakness

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Barry E. Levin, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.2 |
| Other: | 0.0 |

Project Description:

Objectives: To define more clearly and treat those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis. Attention is directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the muscle fiber: sarcolemma, T-system, sarcoplasmic reticulum, myofibrillar complex (i.e., the excitation-contraction coupling mechanism). Studies are done with agents which are either provocative or therapeutic with respect to periodic paralysis syndromes, with a view to obtaining more information regarding pertinent metabolic pathways and methods of treatment.

Methods Employed: The techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electron microscopy, and biochemical assays of tissue are delineated in our other projects. Provocation loading tests and therapeutic trials to raise or lower potassium or sodium were used. Acetazolamide was administered as a prophylactic agent for hypokalemic periodic paralysis.

Patient Material: Patients of all ages are admitted to the Medical Neurology Branch for this project if they have: intermittent muscular weakness associated with familial periodic paralysis, hypo- or hyperkalemic; isolated examples of periodic paralysis with potassium disturbances; thyrotoxic periodic paralysis; paramyotonia congenita; or myotonic congenita. (Patients with myasthenia gravis are part of another Medical Neurology Branch Project.)

Major Findings: A new use for acetazolamide (Diamox), a carbonic anhydrase inhibitor, was introduced by us 7 years ago -- it is now widely accepted as the best prophylactic agent available in hypokalemic periodic paralysis. Our on-going follow-up discloses that it has thus far provided moderate to striking benefit for 19 of our 21 patients, two for over 7 years. It has also improved the moderate or severe persistent proximal weakness between attacks that afflicted 16 patients on convential therapy. The mechanisms of acetazolamide action in this disease remains unknown, since muscle itself has no carbonic anhydrase.

Significance to Bio-Medical Research and the Program of the Institute: Acetazolamide has proven to be the most effective single prophylactic agent available in the treatment of hypokalemic periodic paralysis. It provides new avenues for investigation of the basic pathophysiology in this disease.

Proposed Course of Project: To explore in more detail, with patients and animals, the mechanism of action of acetazolamide prophylaxis in hypokalemic periodic paralysis and the pathogenesis of the disease itself. To seek even more effective therapeutic agents.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-65 MN/OC 1190(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Myasthenia Gravis (MG)

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Bernard M. Patten, M.D.
John R. Warmolts, M.D.

Other Investigators: Peter K. Re', M.D.
John N. Whitaker, M.D.
Gabriel Genkins, M.D.

Cooperating Units: Department of Medicine, Mt. Sinai Hospital, New York, N.Y.

Man Years:

| | |
|---|---|
| Total: | 1.2 |
| Professional: | 1.0 |
| Other: | 0.2 |

Project Description:

Objectives: To apply histochemical, pharmacologic, electrophysiologic and immunologic techniques to investigate the pathogenesis of myasthenia gravis. To seek new or improved methods of treatment.

Methods Employed: Clinical studies, including edrophonium, prostigmine and curare challenging tests, were done on patients with myasthenia gravis, on a group of patients with myasthenic syndrome in association with clinical entities with established denervation, and on a number of patients with a variety of other neuromuscular diseases. Competence of neuromuscular transmission was studied neurophysiologically in patients with myasthenia gravis and other neuromuscular disorders. Experience was extended and modified with a new treatment program introduced by us, consisting of high-single-dose (100 mg for the average adult) alternate-day prednisone with adjuncts of supplemental potassium, antacids, and low sodium, low carbohydrate and high protein diet. A rat nerve-muscle preparation was used to seek (a) possible adverse interaction between anticholinesterase and anticholinesterase drugs; and (b) an inhibitory factor released by MG thymus tissue during 24 hour culture *in vitro*.

Patient Material: Myasthenia gravis patients participated in the therapeutic trials. Sera, muscle, thymus and other tissue were obtained during diagnostic or therapeutic procedures from Medical Neurology Branch patients, and thymus was obtained from therapeutic thymectomies done on myasthenia gravis patients at the Mount Sinai Hospital, New York, N.Y.

Major Findings: The complete hypothesis utilizing data from many types of investigative studies we reported previously, to the effect that myasthenia gravis (MG) is a disorder of the lower motoneuron soma, which fails to provide a qualitatively and/or quantitatively adequate axoplasmic flow to nourish the axon tips, continues to be our explanation of choice for the pathogenesis of MG. This motoneuron defect is proposed as being secondary to an abnormality of thymus and related tissues. Certain known "immunologic" alterations in MG are by this hypothesis considered parallel and potentially informative phenomena but not pathogenic of the muscle weakness. The motoneuron abnormality is viewed as initially causing a greater defect of excitation factor (acetylcholine) and later a diminution of "trophic factor" too.

A male with juvenile myasthenia gravis has now been maintained at about 95% improvement for 6 years on corticosteroids, the last 3 1/2 years on the program new for this disease, high-single-dose alternate-day prednisone. A second juvenile myasthenic male has also shown beneficial response for 40 months, and a third male for 18 months. This is in contrast to the short course of ACTH customarily given for adult myasthenia gravis. No juvenile has failed to respond.

The new treatment that we introduced for adult myasthenia gravis, namely high-single-dose alternate-day prednisone has continued to give excellent results in 14 of 16 adult patients thus far treated, proving to be much more effective therapy than anticholinesterase drugs (which we prefer to discontinue when prednisone is begun). It is beneficial in patients with or without previous thymectomy. Our only two prednisone failures were females of menstruating age, but our series is not yet large enough to establish for certain which MG patients are most likely to respond. Many others throughout the United States and the world have confirmed our beneficial results with prednisone in MG.

Clinical impression has suggested an adverse interaction between corticosteroid (prednisone) and anticholinesterase (pyridostigmine or neostigmine). Accordingly, such an effect was sought and found with a rat nerve-muscle test preparation. Steroid (hydrocortisone) abolished twitch-augmenting effects of pyridostigmine and neostigmine, and once steroid had abolished the anticholinesterase effect equivalent doses of the anticholinesterase were less effective than before steroid had been given. In water-bath experiments using frog rectus muscle it was shown that prednisolone and hydrocortisone in concentrations of 0.4 micromole/ml can block contractions induced by acetylcholine; steroid hormones had 1/10th the blocking activity, in this preparation, as d-tubocurarine. If these findings relate to the situation in the myasthenic patient they explain why some myasthenics taking corticosteroids and anticholinesterases may do much worse than those taking steroid alone.

In collaboration with Dr. Genkins at Mount Sinai Hospital, we have demonstrated that 6 of 10 myasthenic thymuses in tissue culture make a powerful nerve-muscle blocking agent demonstrable in a rat nerve-muscle preparation. It resembles curare but has several properties distinctly different from curare. Seven control thymuses processed in the same way produced little or no blocking activity. Future work would aim to isolate, synthesize, and neutralize this thymus produced factor.

We had previously shown that the serum antimuscle antibody characteristic of MG is an immunoglobulin G and a true antibody. Using electromicroscopy and immunolocalization with peroxidase labeled reagents, we have now shown that that antibody localizes to the sarcoplasmic reticulum but exclusively at the I-band level. This is the first demonstration of a chemical difference between the reticulum at the I vs. A bands. (See our Electronmicroscopy Project.)

A serum antibody specifically against neuronal nuclei but not against nuclei of other cells (except spermatogonia) was reported in MG by another group. Our studies fail to reveal such a specific antineuronal antibody in sera of many myasthenics representing various ages and duration and severity of disease, making us skeptical of the other initial report.

Significance to Bio-Medical Research and the Program of the Institute: The response of myasthenic patients to alternate-day prednisone indicates that much of the abnormality is not irreversible and supports the idea that improvement can result even in the most severely affected and advanced stages of this disease. The demonstrated benefit of alternate-day prednisone in juvenile myasthenia gravis led us to apply this new program to adult myasthenia gravis patients. It has resulted in a remarkable new method of treatment, using a drug that has been and is still listed in some souces as "contra-indicated" in myasthenia gravis. Advantages of this prednisone program in the treatment of myasthenia gravis include its being a nonsurgical means of providing lasting improvement by an oral medication not requiring administration at rigid intervals during the day, not producing an initial drug-induced worsening, allowing continuous long-term use, and with its effect thought to be anti-pathogenic rather than anti-symptomatic. We now consider alternate-day prednisone the most effective treatment in myasthenia gravis and believe it offers the possibility of suppressing the disease effectively for a sufficiently long period for it to disappear completely. If this will occur, the disease would be cured in such a patient.

The adverse interaction between steroid hormone and anticholinesterase drug, since it parallels clinical impression, dictates caution when one contemplates their combined use clinically, although it is conceivable that in the MG disease state the two in certain combinations might allow the beneficial of each to outweight their interacting detrimental effect.

Since many suspect the thymus (and other similar tissues) of producing a substance detrimental directly or indirectly to the neuromuscular junction, our finding such an effector released from incubated MG thymic tissue opens the way for its precise chemical identification, cell of origin, cause of production, and means of prevention.

Proposed Course of Project: The various clinical and basic mechanistic aspects of our new alternate-day prednisone program will be investigated more extensively. The detrimental substance(s) from the MG thymus will be explored in more detail.

Honors and Awards: None

Publications:

Engel, W. K. and Warmolts, J. R.: Myasthenia gravis successfully treated with long-term high-single-dose alternate-day (HSDAD) prednisone. The 10th Intern. Cong. of Neurol. Barcelona, Spain, 1973. In press.

Patten, B. M. and Oliver, K. L.: Adverse interaction between steroid hormones and anticholinesterase drugs. Trans. Amer. Neurol. Assn. 1973. In press.

Serial No. NDS(I)-65 MN/OC 1191(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunological Abnormalities of Neurologic Diseases

Previous Serial Number: Same

Principal Investigators: John N. Whitaker, M.D.
W. King Engel, M.D.

Other Investigators:
John L. Sever, M.D.
Darell D. Bigner, M.D.
Warren Strober, M.D.
Peter K. Re', M.D.
P. V. Holland, M.D.
H. J. Alter, M.D.
Sidney F. Stein, M.D.
Robert D. Gerwin, M.D.
Barry E. Levin, M.D.
Richard W. Price, M.D.

Cooperating Units:
Department of Neurology, V. A. Hospital, Minneapolis
Perinatal Research Branch, NINDS
Metabolism Branch, NCI
Blood Bank, Clinical Center
Laboratory of Clinical Investigation, NIAID

Man Years:

| | |
|---|---|
| Total: | 1.4 |
| Professional: | 1.0 |
| Other: | 0.4 |

Project Description:

Objectives: To apply immunological techniques to the study of diseases affecting the central nervous system and neuromuscular system.

Methods Employed: Various immunochemical techniques were employed for studying both immunoglobulin and cellular aspects of altered immune states.

Patient Material: Specimens of peripheral blood, urine, cerebrospinal fluid (CSF), bone marrow, muscle, peripheral nerve, thymus and other tissue was obtained from patients with neurological disease admitted to the Medical Neurology Branch.

Major Findings: Subacute sclerosing panencephalitis (SSPE) has come to be considered an effect of the rubeola virus -- a lingering infection with a "slow" form of the virus or an altered immune response to it, or both. We have now reported SSPE in only one of identical twins, documented immunologically (with PR-CFR). Two treatment attempts using transfer factor from the patient's healthy twin were not successful (with PR-CFR, NCI). This evidence tends to downgrade certain suspected etiologic variables, e.g., host genetic composition, strain of rubeola virus, or general environmental factors, but is still compatible with others, e.g., prior or subsequent infections with other viruses or genetic composition other than that of the tested major histocompatibility loci.

Specimens collected from every Medical Neurology Branch patient and stored in the serum and spinal fluid bank have proven to be valuable in the study of a number of entities of possible viral and/or autoimmune etiology.

Our finding of immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases, reported last year, has now been confirmed by others. This supported our earlier hypothesis that childhood polymyositis/dermatomyositis (PM/DM) may be another form of blood vessel abnormality in muscle, but on the venous side. Since this and other immunologic abnormalities of PM/DM could in turn be caused by a viral infection, search for Australian antigen, circulating or bound to blood vessels or muscle cells, was made but proved completely negative (with CC).

In order to analyse the effect of direct viral involvement of muscle tissue, a model of experimental viral myositis is being established.

Serum immunoglobulin and $B_{1C/1A}$-globulin levels have been determined on a large group of patients from the Medical Neurology Branch. A number of patients with a chronic peripheral neuropathy currently considered to be idiopathic were found to have an elevated immunoglobulin M, suggesting an immunologic pathogenesis in them.

A very interesting finding was that 6 of 7 patients of a family with hereditary sensory neuropathy had elevated immunoglobulin A in their serum. Studies to determine the cause of the increased levels of serum immunoglobulin A were done in collaboration with the Metabolism Branch, NCI. Three of four patients affected with the hereditary sensory neuropathy had elevated small bowel production of immunoglobulin A; one affected patient and two unaffected siblings had normal small bowel immunoglobulin A production levels. Although this finding does not at the present time explain the cause of the neuropathy, it may add significant information to understanding possible pathogenetic mechanisms and has prompted a new hypothesis concerning the mechanism of their neuropathy: possible familial susceptibility to infection in the gut tissue (in which there are excessive eosinophils, suggesting a local immunologic reaction there) that causes through, or parallel to, the IgA overproduction a metabolic abnormality affecting sensory nerves (see our Amyotrophic Lateral Sclerosis Project).

As part of the same study in which immunoglobulin and complement levels were studied in patients, immunoelectrophoretic determinations had been done on all the patients admitted to the Medical Neurology Branch. In one patient with adult-onset rod neuromuscular disease, an unusual paraproteinemia was found consisting of a IgG/$\lambda$ myeloma protein. He appears to have had a monoclonal gammopathy for over a year, without detectable evidence of myeloma. The possible relationship between the paraprotein and the neuromuscular disease is being explored. In his biopsy, we recently found rods in the muscle nuclei that are ultrastructurally identical to those within myofibrils.

The site of the abnormality in myasthenia gravis is still undetermined. Evidence has accumulated that the neuron and presynaptic mechanisms may underlie the myasthenia gravis defect. Because of this the studies previously done by another worker, in which he found antineuronal binding of sera from patients with myasthenia gravis, were very important but needed to be confirmed and possibly extended. He suggested that the factor was neuron-specific, binding to nuclei of neurons only, except for spermatogonia binding too. In an indirect immunofluorescent study of 15 patients with myasthenia gravis, binding of antibodies to neurons (to their nuclei) of the IgG, IgA and IgM class was found only in those patients who also had antinuclear antibody in these classes. Additionally, patients with systemic lupus erythematosus, who did not have myasthenia gravis but who did have antinuclear factor, had the same spectrum of staining patterns. Therefore, we conclude that the neuronal nuclear binding really represents part of the antinuclear factor activity sometimes present in the sera of patients with myasthenia gravis and is neither neuronal-specific nor a phenomenon specific to that disease.

The binding site of the anti-muscle factor highly characteristic of myasthenia gravis (MG) (which we previously showed was an IgG and true antibody), was localized with immunohistochemistry-electron microscopy to the sarcoplasmic reticulum but only at the I-band level. Incidentally, this demonstrates chemical specialization of the reticulum of the I-band in contrast to that of the A-band. It does not immediately explain the cause of the severe weakness of MG, but provides another avenue to explore (see our Electronmicroscopy Project).

Tissue from thymuses of 5 of 8 MG patients during 24-48 hours in tissue culture liberated a substance(s) strongly inhibitory to an animal nerve-muscle test preparation (see our Myasthenia Gravis Project). The chemical nature and method of action of that substance must now be defined.

In order to analyse adrenergic nerve endings and the role of their possible alteration in vascular diseases of the brain and muscle, specific antibodies are being made to dopamine-β-hydroxylase, because the immunologic method of demonstrating those nerve endings is more specific and more distinct than the catecholamine fluorescence method, which will be done in parallel as a complementary analytical technique.

Finding an identical twin unaffected by subacute sclerosing panencephalitis but living in the same environment as her severely affected sister indicates need for consideration of predisposing factors in addition to the rubeola virus itself, some of which are reviewed above.

The various immunoglobulin abnormalities found in a family with hereditary sensory neuropathy and in some of our other neuromuscular disease patients provoke new ideas regarding possible immunologic abnormalities that may be operative in the pathogenesis of these diseases -- they may also provide leads to treatment.

Proposed Course of Project: Ideally, all the immunologic studies noted herein should be elucidated more fully with the best available immunological techniques. However, we do not have a position to use for an immunologic investigator or for a technician. Therefore the course of the project is somewhat uncertain.

Honors and Awards: None

Publications:

Whitaker, J. N., Sever, J. L. and Engel, W. K.: Subacute sclerosing panencephalitis in only one of identical twins. N. Engl. J. Med. 287: 864-866, 1972.

Whitaker, J. N., Holland, P. V., Alter, H. J. and Engel, W. K.: Idiopathic inflammatory myopathy. Arch. Neurol. 1973. In press.

Gerwin, R. D., Hadler, N. M., Frank, M. M. and Engel, W. K.: The fourth component of complement in the cerebrospinal fluid in systemic lupus erythematosus. Neurology 23: 411, 1973.

Whitaker, J. N., Sciabbarresi, J., Engel, W. K., Warmolts, J. R. and Strober, W.: Serum immunoglobulin and complement (C3) levels in adults with idiopathic chronic polyneuropathies and motor neuron diseases. Neurology 23: 425, 1973.

Whitaker, J. N., Falchuck, A. M., Blaese, R. M., Engel, W. K. and Strober, W.: Disorders of the immunoglobulin A system in hereditary sensory neuropathy. Arch. Neurol. 1973. In press.

Serial No. NDS(I)-65 MN/OC 1192(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electron Microscopic Studies of Skeletal Muscle and Neurons

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Jerry R. Mendell, M.D.

Other Investigators: Mary Ann Oberc, B.A.
Robert I. Roelofs, M.D.
John N. Whitaker, M.D.
Ewa Sawicka, M.D.
Husseiny I. Dessouky, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 3.1 |
| Professional: | 2.1 |
| Other: | 1.0 |

Project Description:

Objectives: To study the early subcellular changes of human muscle in diseases that are confined to muscle alone, as well as in conditions which produce secondary alterations in muscle, such as denervating and metabolic diseases. To study subcellular changes of human neurons and peripheral nerves in certain diseases.

Methods Employed: A portion of biopsied muscle is placed in our specially-designed clamp to maintain resting length and is immediately fixed in cold buffered glutaraldehyde or osmium tetroxide for eventual araldite embedding. Other fixative combinations, buffers (including cacodylate), and various electron microscopic (EM) staining procedures are used. EM histochemistry for acid phosphatase, cytochrome oxidase, succinic dehydrogenase, and an ATPase have been done on human muscle. Adjacent biopsy material is immediately frozen for cryostat sections and incubated in various histochemical media, as described in the Histochemistry Project. Histochemically stained cryostat sections, ready within half an hour after the biopsy, are used to determine which of the biopsies contain light microscopic features of special interest and thereby guide choosing material appropriate for electron microscopic study and for directing the search for ultrastructural abnormalities. If necessary, a repeat biopsy of a patient can be done by needle technique to obtain additional tissue for EM. Biopsy specimens of human nerve or brain tissue are fixed and embedded for electron microscopy and are similarly monitored by rapid enzyme histochemistry of adjacent tissue. Skin samples and cultured fibroblasts from our patients are sometimes studied as are mitochondrial preps of sarcolemmal membrane from our biochemical studies. In the animal models studied in parallel to the human diseases, tracer substances of peroxidase, ferritin, and lanthanum are sometimes used with electron microscopy.

Patient Material: Patients with various myopathies and neurogenic muscular weakness are biopsied for diagnostic purposes. When available, tissue from asymptomatic carriers of progressive genetic myopathies is taken for study. Muscle biopsies and nerve biopsies are the major tissues examined. Muscle from patients is sometimes grown in tissue culture for a few weeks before being studied by EM. Occasional brain biopsies are obtained from patients with certain forms of progressive degenerative disease. Other abnormal tissues from neurologic patients are occasionally examined, such as skin and cultured fibroblasts.

Major Findings: In myasthenia gravis, the precise binding site of the highly characteristic serum muscle-binding factor (Strauss, et al.), which we have previously shown to be an immunoglobulin G and true antibody, was localized by electron microscopy-immunology. Using the indirect Coors technique and peroxidase-labeled reagent, it was found that the serum anti-muscle factor binds only to the sarcoplasmic reticulum of the I-band in the muscle fiber, and not to the reticulum of the A-band. This is the first demonstration of a structural chemical difference between the reticulum of the I vs. A band.

The first EM studies of cultured normal human skeletal muscle were published. It was found that the fibers grown in vitro without nerve supply undergo advanced cellular differentiation in regard to all components except the T-tubules and sarcoplasmic, which are very scant. Their paucity may be the anatomical explanation for the observation that human, in contrast to chick, fibers in culture do not contract spontaneously (see our Tissue Culture Project).

EM studies of cultured muscle from patients with various neuromuscular diseases are in progress.

A new structural abnormality of human muscle fibers has been identified by light-microscopic histochemistry and by EM. It is tentatively called "zipper tubules" and exists only in the type I fibers.

In what may be a newly recognized clinical form of human acid-maltase deficiency, a juvenile chronic type, the accumulated excess glycogen was shown by EM to be both within and outside of membrane-bound structures.

The second (our first) well-studied case of a grave muscle and cardiac involvement in a disease characterized by accumulation in the muscle fibers of a bizzare PAS-positive diastase-resistant polysaccharide was studied by EM. The accumulated material is not glycogen granules but filamentous forms. Their biochemical nature is yet unknown but being investigated.

Attempts are being made to localize at the EM level the acid-preincubated ATPase reaction of skeletal muscle fibers, which we have previously introduced to light microscopy. Preliminary results suggest it is localized in the sarcoplasmic reticulum at the I-band level with emphasis at the A-band-I-band junction.

In a new patient with late-onset rod disease (an electron microscopically delineated entity we have originally described previously), who incidentally was found to have a monoclonal gammopathy (IgG/$\lambda$ myeloma protein in the serum), we have delineated by EM intranuclear rods morphologically identical to those of the cytoplasm.

"Ragged red fibers" is a now generally used term we introduced to describe the histochemical appearance of abnormal muscle fibers which contain collections of large, bizzarely-structured mitochondria by EM. We have now observed such fibers in a new hereditary syndrome consisting of limb-girdle dystrophy, myoclonus epilepsy and elevated basal lactates and pyruvates that can be fatal in childhood (see our Myopathy Project).

Histochemical techniques for cytochrome oxidase, succinic dehydrogenase, and acid phosphatase have now been established in the laboratory and are being used on the human biopsies. The initial objective is to delineate the bizzare mitochondrial changes present in "ragged red fibers."

Electron micrographs have been made on a number of our other patients and are being correlated with all our other studies.

We have begun to seek and find end-plates in our EM studies of human muscle. First pictures suggest some may be abnormal in central core disease.

An EM search for viral particles in polymyositis and other sporadic neuromuscular diseases is being conducted, in an attempt to confirm one report by another group. To date, "myxovirus-like" structures have been found in some cases of polymyositis, but their significance remains unknown.

Significance to Bio-Medical Research and the Program of the Institute: Analysis of the changes in ultrastructure of the neuromuscular disorders is in its childhood. Identification of new forms of disease still occur rapidly in this stage. However, one must be careful not to name a condition on the basis of limited, though careful, EM or histochemical studies. Therefore, a broad experience with as many well-studied cases as possible is necessary, as close correlation of EM with histochemistry. It appears that certain morphologic changes can indicate suspected metabolic defects, as in crystalline and other changes of mitochondria. Certain other ultrastructural changes, e.g., accumulation of lipid or lysomal glycogen in fibers provide clues as to which metabolic pathways are likely to be the site of a presumed enzyme defect. These ultrastructural changes will help guide formulation of pathogenesis and treatment of the neuromuscular and neurologic disorders. Experimental production of identical defects provides a new tool for analysing these changes. Similar expectations pertain to the other human neuromuscular disorders being studied, as well as the neuronal abnormalities. Electron microscopy is also indispensible for the detailed morphologic analysis of topics listed under our other projects, e.g., the viral-etiology hypothesis of animal and human brain tumors and the possible viral etiology of polymyositis and dermatomyositis.

Proposed Course of Project: Further studies that combine histochemical, immunologic, autoradiographic, viral, and tissue culture techniques with electron microscopy applied to human neurologic diseases and animal models have been planned, so as to supplement the morphologic approach with pertinent studies of cellular dynamics.

Honors and Awards: None

Publications:

Mendell, J. R., Whitaker, J. N. and Engel, W. K.: The skeletal muscle binding site of anti-striated muscle antibody: An ultrastructural study using peroxidase conjugated antibody. J. Neuropathol. Exp. Neurol. 30: 233, 1972.

Mendell, J. R., Whitaker, J. N. and Engel, W. K.: The skeletal muscle binding site of anti-striated muscle antibody: An ultrastructural study using peroxidase conjugated antibody. J. Immunol. 1973. In press.

Serial No. NDS(I)-65 MN/OC 1193(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Radioautography Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Barry W. Festoff, M.D.

Other Investigators: Malcolm C. Johnston, M.D.

Cooperating Units: Human Genetics Branch, NIDR

Man Years:

Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:

Objectives: To apply radioautographic techniques to study structure and function in normal and abnormal neurons and muscle cells of man and animals.

Methods Employed: Standard radioautographic methods, primarily using emulsion-dipped slides. Various histochemical enzymatic counterstains for end-plate and muscle fiber components are done after photographic development of the slides.

Patient Material: Muscle biopsies obtained from our patients for diagnostic purposes -- additional sections are cut from the blocks for incubation with various radioisotopes, e.g., $^{125}I$-$_{\alpha}$-bungarotoxin.

Major Findings: A method has been obtained for demonstrating localization of $^{125}I$-$_{\alpha}$-bungarotoxin (which we have highly purified) to the cholinergic receptor molecules neuromuscular junction which is made manifest by histochemical post-staining for acetylcholinesterase. This technique is now being applied to various human diseases known or suspected to be related to abnormality (a) at the neuromuscular junction or (b) of the nerve influence on muscle. The latter is expected to allow spread of $_{\alpha}$-bungarotoxin receptors away from the normal narrow confines of the neuromuscular junction.

Significance to Bio-Medical Research and the Program of the Institute: Several human neuromuscular diseases are still contraversial regarding their neurogenic vs. myogenic pathogenesis. The technique might contribute to clarification of that most essential point.

Proposed Course of Project: Extend our primary studies to various human neuromuscular diseases in a more complete and systematic manner. Ideally, we would like to obtain an additional room and a technician to do all the work in our own Branch.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 MN/OC 1728(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Brain Tumor

Previous Serial Number: Same

Principal Investigators: Darell D. Bigner, M.D.
John P. Kvedar, A.B.
W. King Engel, M.D.
Nicholas A. Vick, M.D.

Other Investigators: W. Ray Bryan, Ph.D.

Cooperating Units: Viral Oncology Branch, NCI
Duke University School of Medicine,
Department of Neurology, Durham, N.C.

Man Years:

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|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives: (1) To develop a brain tumor animal model in which to study the pathogenesis and better methods of diagnosis of brain tumors; (2) to develop the most sensitive animal system in which to attempt recovery of oncogenic viruses from human brain tumors; (3) to improve methods of virus extraction from SR-Rous sarcoma virus (SR-RSV) tumors; and (4) to develop new methods of treating brain tumors.

Methods Employed: (a) Assay of SR-RSV, an RNA tumor-inducing virus, on the chorioallantoic membrane of chicken eggs; (b) preparation of high-potency, bacterial-free suspensions of SR-RSV by a new combination of buffer systems, differential centrifugation, and membrane filtration; (c) induction of brain tumors in young dogs or hamsters by injecting the high-potency suspensions of SR-RSV; (d) measurement of antibody responses in tumor-bearing dogs, demonstration of SR-RSV antigen in the induced brain tumors; (e) recovery of SR-RSV from the tumors in chick embryo fibroblast tissue culture, in chicken, or in

Japanese quail; (f) measurement of the effect of various drugs on reducing the amount of SR-RSV necessary from brain tumor induction; (g) morphologic study with histochemical, electron microscopic, light microscopic, and immunofluorescent techniques, seeking (1) viral particles and (2) details of the morphologic changes of the neoplastic cells; (h) brain scanning of tumor-bearing animals with various isotopes.

Patient Material: None

Major Findings: Published this year was a detailed account of this work, covering the following data. An improved method of concentrating and purifying Schmidt-Ruppin Rous avian sarcoma virus (SR-RSV) yielded, 4 weeks after intracerebral inoculation into newborn dogs and hamsters, 96% survivor incidence and 100% brain tumor incidence in survivors, compared to 49% and 86% respectively with previous methods. A quantitative dose-response relationship was demonstrated. The tumors were sarcomas and gliomas (spongioblastomas, astrocytomas, and ependymomas) with varying degrees of anaplasia. With a smaller (0.01 ml) inoculum volume of the most highly concentrated SR-RSV, brain inoculation site was the determinant of type of tumor induced; 7/10 dogs inoculated in the subependymal cell plate developed only gliomas, and all dogs in which tumors were induced after inoculation in the parietal subdural space (5/5) or the cerebellar vermis (4/5) developed sarcomas. No evidence of virus was found in dog brain tumors with electron microscopy or immunologic methods, but the SR-RSV genome was detected by inoculating whole cell suspension of dog and hamster brain sarcomas and gliomas back into avian hosts, wherein typical Rous sarcomas containing C-type viral particles, group specific viral antigens, and infectious RSV were induced.

Significance to Bio-Medical Research and the Program of the Institute: These induced brain tumors are an especially suitable system in which to study mechanisms that allow malignant transformation of glial and mesodermal brain cells by RNA-C-type viruses without detectable evidence of viral replication, as is likely to be occurring in at least some spontaneous human brain tumors. This system will serve as a guide to isolation of viruses causing human brain tumors. The reproducibility and rapidity of experimental tumor induction, type of blood supply, and histologic similarity of the experimental gliomas to spontaneous human gliomas make these SR-RSV-induced animal brain tumors ideal for morphologic, chemotherapeutic and brain-scanning studies. They are now being used to study (with LNNS, IR) the blood-brain-barrier changes in tumors, combining tracer substances with electron microscopy.

Perhaps the most important implication is the fact that virally induced brain tumors that have no evidence of viral particles or viral antigens, but which do contain viral genetic material, are available for study, since these same conditions may be operative in spontaneous human brain tumors.

Proposed Course of Project: Ideally the project should be continued in several directions. First, the technology of concentrating SR-RSV should be extended to extraction of spontaneous human brain tumors for inoculation of animals to try to demonstrate the viral eitology of human tumors. In addition, the basic cellular mechanisms that allow malignant transformation of cells with our detectable evidence of viral antigens or mature virus should be studied and applied to the human situation. Also, the ability to induce large brain tumors in large and small animals should be exploited for evaluating chemotherapeutic agents and better diagnostic methods, such as brain scanning.

However, since neither positions nor space has been granted for this project, it must remain dormant in this Branch.

Honors and Awards: None

Publications:

Bigner, D. D., Kvedar, J. P., Shaffer, T. C., Vick, N. A., Engel, W. K. and Day, E. D.: Factors influencing the cell type of brain tumors induced in dogs by Schmidt-Ruppin Rous sarcoma virus. *J. Neuropathol. Exp. Neurol.* 31: 583-595, 1972.

Serial No. NDS(I)-70 MN/OC 1792(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electrophysiology Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
John R. Warmolts, M.D.

Other Investigators: Alan D. Prince, M.D.
M. Zafar Mahmud, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.7 |
| Professional: | 1.7 |
| Other: | 0.0 |

Project Description:

Objectives: (1) To develop more precise methods of electromyography (EMG) recording and analysis; (2) to establish better EMG indices of early myopathic or neuropathic involvement; (3) to improve the value of the EMG in distinguishing the neural vs. myopathic basis of atypical disorders; (4) to investigate mechanisms regulating motor unit recruitment in normal subjects and those with neuromuscular disorders; (5) to correlate the electrical behavior of motor units with their histochemically defined properties; (6) to use clinical electrophysiologic parameters to evaluate course of disease and response to treatment.

Methods Employed: Recordings are made in humans using the Teca-Medelec electrophysiologic system. Permanent data are stored by high-speed magnetic tape recording and photographic recording. Correlation studies involve the (a) recruitment threshold, (b) rank order of recruitment, (c) preferential stable discharge frequency, (d) relative rhythmicity, (e) amplitude, (f) duration, and (g) configuration of EMG recorded individual motor unit action potentials in human subjects with a variety of neuropathic and myopathic conditions, and with neuromuscular disease of uncertain type, as well as in subjects without definable neuromuscular disease. A method was developed for EMG recording in the operating room during open biopsy and excising the region of fibers directly around the tip of the EMG electrode ("Open Biopsy EMG").

A variety of parameters of neuromuscular transmission are repeatedly evaluated in individual myasthenia gravis patients before and during treatment. Partial curarization of subjects with various fatigue states have been performed with attention directed primarily to the behavior of single motor units.

Electrophysiologic abnormalities were sought in various systemic diseases affecting the neuromuscular system, such as hyperparathyroidism and non-metastatic distant cancer, and correlated with clinical and histochemical data.

Patient Material: Patients with a variety of neuromuscular disorders and without definable neuromuscular disease from the Medical Neurology Branch and the neurologic consultation service we provide throughout the Clinical Center, in whom diagnostic EMG studies are being done.

Major Findings: Five new aspects of clinical electromyography (EMG) have been published: (a) A new descriptive term for the pattern of short duration, small amplitude, excessively abundant potentials for a given amount of voluntary effort, viz., "SSAP", was introduced to replace the generally used term "myopathic", a designation which we consider to be misleading at times. We consider SSAPs just as likely to be due to neuronal disease as to myopathic disease. (b) Published was the new technique of "Open Biopsy Electromyography" we have introduced to provide tight correlation of electrophysiology done by EMG recording of the same muscle fibers excised immediately after recording and studied histochemically. (c) Using this technique in patients with biopsies showing predominance of either type I or type II muscle fibers, we have identified the different physiological properties of these two human motor unit types. Type I motor units fire with mild initial effort and discharge more regularly and more continuously, while type II units fire only irregularly in unsustained bursts and only when more vigorous contraction is exerted. (d) The electromyographic patterns of selective type II and type I muscle fiber atrophy in humans have been identified, and an hypothesis developed that relates the electrical data to the neurogenic origin we propose for these atrophies. (e) Morphologic confirmation of motor end-plates as the signal source for "end-plate noise" has been achieved in human limb muscle through open-biopsy EMG.

A fixed order of recruitment of motor unit action potentials (MUAPs) from motor units during sustained isometric limb contractions in both proximal and distal, upper and lower limb muscles that is dependent upon the strength of voluntary contraction has been published. Succeeding MUAPs appearing on graduated isometric contraction demonstrate respectively increasing amplitude and duration. Variations from this occur when action potentials of different motor units are of very near size and threshold. No difference in stable discharge rhythmicity has been correlated with order of recruitment or size among the first several motor unit potentials recruited at any one recording point in muscle.

On the basis of correlated electromyographic and histochemical study of certain diseases and in comparison to theoretical possibilities, we have proposed that what is currently termed a "myopathic electromyogram (EMG)" (an "SSAP pattern" in our terminology) might in several instances be caused by abnormality of the lower motor neuron or its axonal branches. We have formulated several different theoretical neuropathic mechanisms that could cause an SSAP pattern. Disease examples are myasthenia gravis, central core disease, myotonic atrophy (formerly called myotonic dystrophy), myotonia congenita, type-I-fiber-hypotrophy-with-central-nuclei, type-I-fiber-smallness-without-central nuclei, and the form of benign congenital hypotonia with type I fiber predominance (see our ALS and Myopathies Projects). Thus the overly simplified designation by many of "myopathic EMG" or "the EMG was myopathic" is in some instances certainly not proved to be non-neurogenic and in theoretical liklihood probably is, according to our best guess at this time.

Using a micromarking technique we adapted for open biopsy electromyography, we have verified in human limb muscle that motor end-plates are the signal source for what has been postulated to be "end-plate noise", through the use of cholinesterase staining of structures marked after their emanating "end-plate noise" was recorded. With this technique, studies of microscopic morphology as correlated with cholinesterase-staining, histochemical fiber type, and nerve terminal proliferation as demonstrated by axon silver staining, can be correlated in normal humans and ones having various neuromuscular diseases with behavior of miniature end-plate potential activity in response to pharmacologic agents.

It was published last year and documented further that detailed and repeated electrophysiologic measurements on myasthenic patients treated with our newly-introduced program of long-term high-single-dose alternate-day prednisone clearly documented both a 48 hour cyclic and more sustained cumulative benefit of prednisone on muscle strength and endurance. For example, in one patient the improvement in adductor pollicis action potential response to repetitive nerve stimulation during the evening of on-prednisone days, as compared with off-prednisone days, indicated that maximal benefit occurred during the prednisone day. In another patient, comparison of the amplitudes of the 2 orbicularis oris responses to twin shocks delivered to the facial nerve at intervals of from 10 msec to 1,000 msec, demonstrated increased synaptic efficiency at each inter-shock interval during the evening of the prednisone-treatment day as compared to that morning before receiving prednisone. Both effects were best demonstrated during the initial days of treatment of a third patient. Lessening fall-off of amplitude of abductor digiti quinti response to twin ulnar nerve supramaximal shocks 300 msec apart showed that improved neuromuscular synaptic efficiency could be detected within several hours of the 100 mg oral prednisone dose, reached its peak at around 12 hours after the dose, and then ebbed to a level of overall improved function until a succeeding 48 hour cycle of improvement followed the next prednisone dose. The electrical improvement correlated directly with improved clinical strength in this and other involved muscles. This 48 hour cycle, being in phase with prednisone administration but not with any other medication or environmental variable, is convincing evidence that prednisone therapy is responsible for improvement. This also paralleled clinical evidence of a 48-hour cyclic response in muscle strength. During the early stage of treatment, strength in involved muscles during both the "peak" and "ebb" phases of each 48 hour cycle was greater than during the preceding 48 hour cycle, resulting in a continued net improvement. Later the rate of improvement was more gradual.

As reported last year, our recent evidence continues to indicate that the studies based on electrophysiology, combined with histochemical and clinical studies support our theoretical formulation of the basis of the clinical improvement in myasthenia gravis with high-single-dose alternate-day prednisone, as follows: rapid improvement initially is based on improvement of the defect of motor nerve ending excitatory function, which may or may not represent correction of a defect in neuronal axoplasmic flow. Intermediate improvement may reflect physical repair of nerve endings and the post-synaptic region of the muscle fiber. Later improvement may be based on return toward normal of atrophied muscle fibers.

In a number of systemic diseases, the presence and type of neuromuscular involvement was identified by EMG correlated with histochemistry. For example, in hyperparathyroidism (with NIAMD), nearly all of 16 patients were considered to have mild to moderately-severe lower motor neuron involvement, sometimes mimicing amyotrophic lateral sclerosis (see our ALS Project). In very early, non-metastatic, distant cancer most patients had slight or moderate evidence of type II fiber atrophy that was, at least in most cases, probably based on motor neuron involvement (with NCI) (see our ALS Project). In xeroderma pigmentosum peripheral neuropathy was identified (with NCI) (see our ALS Project). And, a dysneuronal neuropathy secondary to dapsone therapy was identified, with marked clinical improvement following discontinuation of the drug (with NCI) (see our ALS Project).

Normal standards for voluntary action potentials and nerve conduction studies for this laboratory are being collected.

Significance to Bio-Medical Research and the Program of the Institute: Treatment of human neuromuscular diseases is based on precise diagnosis and understanding of the pathophysiology. As a group, human diseases of the lower motor neurons (including peripheral neuropathies) and muscle are just beginning to have their pathophysiology partly understood. The detailed neurophysiologic techniques herein described are leading to more precise delineation of these diseases, and in some instances causing us to completely change our concepts of their pathogenesis.

EMG is also important for detecting subclinical presence or dissemination of neuromuscular disease, and its type, in patients with various generalized disorders, e.g., distant cancer, hyperparathyroidism, or xeroderma pigmentosum.

We believe that if we can eliminate the term "myopathic EMG" or "the EMG was myopathic" from the neurologic jargon, clinicians and investigators will have a freer approach to think about the possible pathogenesis of various ill-understood neuromuscular diseases.

We are also now learning about different physiological properties of the various types of human motor units, which may lead to a better understanding of their involvement in various neuromuscular diseases.

The use of electrophysiologic criteria to support clinical observations of evaluating our special program of long-term high-single-dose alternate-day prednisone in myasthenia gravis has been extremely useful in our concluding, tentatively, because of our limited number of cases to date, that this now seems to be the single most successful treatment available in myasthenia.

Proposed Course of Project: This project will be continued and elaborated upon, e.g., with even more detailed correlation between EMG and histochemistry in patients with various diseases and further special studies in myasthenia gravis.

Honors and Awards: None

Publications:

Warmolts, J. R. and Engel, W. K.: Open biopsy electromyography. I. Correlation of motor unit behavior with histochemical muscle fiber type in human limb muscle. Arch. Neurol. 27: 512-517, 1972.

Serial No. NDS(I)-62 MN/AP 926(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electromechanical Coupling in Muscle and Drug Activity

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B.S.

Cooperating Units: None

Man Years:

Total: 0.0
Professional: 0.0
Other: 0.0

Project Description:

Objectives: The sequential pattern of physiological events which develop in skeletal muscle starting with excitation and ending in relaxation has not been adequately defined. The molecular interactions that link excitation to contraction in the physiological state are largely unknown. Not much knowledge has accumulated about the molecular changes involved in the uncoupling of contraction from excitation which permits relaxation. The objective was to establish an ionic basis for initiation, maintenance and subsequent loss of tension in slow-type skeletal and in smooth muscle. An additional objective was to relate the ionic events to electrical, mechanical and metabolic activity and to determine how these parameters were related to drug action.

Methods Employed: The coupling and uncoupling processes between the tension-relaxation cycle and the related ionic events are investigated in smooth muscle and in slow-type skeletal muscle. Slow skeletal muscle has now been found to occur in many mammalian species and smooth muscle occurs in all mammals. Since classical electrophysiological and mechanical methods have failed to explain the coupling process ion substitution methods have been developed and used. Sucrose gap techniques have been modified and used for monitoring membrane potential changes. Ion contents are measured by atomic absorption spectrophotometry. Dimonovalent di-buffer amines have been developed and used as monovalent, nonpenetrant, and electrical charge substitutes for Na+ and Cl-.

Major Findings: Last year it was stated that examination of ion content data gathered under conditions of Ca++ lack has verified that increased Na+ entry in slow skeletal relates to the development of contracture tension. This verification is in accordance with the concept that in this type of muscle a Na+ transport system functions to maintain the integrity of muscle function. Similar conditions are probably existent in smooth muscle but more rapid ion transport kinetics have precluded definitions. This year the project has been relatively inactive because the Principal Investigator is on detail as the Assistant Director of the Intramural programs.

Significance to Bio-Medical Research and the Program of the Institute: This project furnishes new data of a basic nature that relates to muscle function and malfunction. Knowledge about smooth muscle may help explain how vascular smooth muscle is involved in stroke mechanisms or the etiology of Duchenne dystrophy.

Proposed Course of Project: This project should continue as long as it yields new knowledge about tissue systems where disease processes occur that are not fully understood. New test systems using vascular smooth muscle can be developed. These could be particularly relevant to determining whether vascular smooth muscle malfunction relates to the onset or course of strokes. The future of this project has not been defined since the Principal Investigator is on detail as the Assistant Director of the Intramural programs.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 MN/AP 1049(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mechanical Properties of Muscle in Relation to Drug Action

Previous Serial Number: Same

Principal Investigator: Jay B. Wells, Ph.D.

Other Investigators: Mark Schoenberg, M.D.
Richard J. Podolsky, Ph.D.

Cooperating Units: Section on Cellular Physics, Laboratory of Physical Biology, NIAMD

Man Years:

| | |
|---|---|
| Total: | 0.7 |
| Professional: | 0.7 |
| Other: | 0.0 |

Project Description:

Objectives: (1) General - The investigation of the fundamental mechanical properties of skeletal muscle was continued in this project as an analysis of a force-generator system and with liberal application of analogue modeling techniques. (2) Specific - Our primary intent was to examine the methods and techniques currently used to estimate muscle elasticity, i.e., the quick-release or controlled-release procedures, with special apparatus tested and described previously in this laboratory. Also, if the current methods were found adequate, we intended to reestimate the elastic constants of active muscle with the improved equipment to establish these values more accurately.

Methods Employed: Estimation of elastic stiffness of active muscle by the current procedures of quick-release or controlled-release proved inadequate therefore a different approach (new methodology) was indicated. The method adopted, the production of acoustical waves and measurement of their propagation rate, is based on the direct relationship existing between such rates and the elastic modulus of the conducting medium. Considerable investigative time was spent on modification and evaluation of apparatus which permitted acquisition of the high speed propagation transients. Therefore, Young's modulus (elastic modulus) has been determined for many isotropic materials (uniform with respect to acoustical propagation rates for axes in any direction) in both liquids and solids. Examples of these materials are, various metals such as steel, brass and aluminum, plastics, glass, water and air. Also tested were the anisotropic materials such as wood, certain polymeric plastics, tendon, muscle and other biologic tissues.

Major Findings: The validity of both method and apparatus was confirmed by comparison of elastic modulus values derived from propagation rates with those obtained, when available, from the technical literature. Based on preliminary measurements, values for elastic stiffness have been obtained for isolated frog muscles at various bath temperatures. As previously reported, temperature change had little effect on the elastic stiffness of the active muscle. The elastic modulus, estimated by the new method, for active sartorius muscle was about ten times greater than that estimated for the same resting muscle. These values are significantly greater than those contained in the literature or obtained from the quick-release method.

Significance to Bio-Medical Research and the Program of the Institute: The work described here is an attempt to apply physical techniques to evaluate particular physical properties of biological tissues. The addition of such techniques to biological research can only result in new information gained for both normal and pathological tissue.

Proposed Course of the Project: Continuation of the project is aimed toward identification of the muscular components which carry the propagated waves and their role in the current scheme of muscular contraction.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 MN/AP 1050(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Functional Activity and Properties of Striated Muscle

Previous Serial Number: Same

Principal Investigator: Jay B. Wells, Ph.D.

Other Investigators: Lloyd Guth, M.D.

Cooperating Units: Laboratory of Neuropathology and Neuroanatomical Sciences, NINDS

Man Years:

| | |
|---|---|
| Total: | 0.3 |
| Professional: | 0.3 |
| Other: | 0.0 |

Project Description:

Objectives: (1) General - This project has continued much as in the past to record, analyze and interpret the changes in mechanical properties and responses of active skeletal muscle which follow specific experimentally produced changes in the "internal" or "external" environment of the muscle. Thus, trophic and adaptive mechanisms are studied which help characterize the normal contractile behavior of voluntary muscle. (2) Specific - This study, which was started in the previous fiscal year with only preliminary findings included in that annual report, was concluded in this fiscal year. We evaluated the effects on histochemical properties and mechanical responses of a changed functional requirement (changed external environment) imposed on soleus muscles in rats. We also evaluated the effects of intramuscularly implanted regenerating alien nerve fibers on normally innervated muscles which was reported to produce trophic changes in histochemistry and contractile speed.

Methods Employed: Standard histochemical techniques and physiological recording of isometric and isotonic contractile responses from in-situ living muscles were used. The functional environment (external) of the muscle was changed by denervation of antagonist muscles and the internal environment was altered in a separate series by intramuscular implantation of the central stump of the cut peroneal nerve.

Major Findings: Modification of the functional environment of the muscle increased the proportion of high-ATPase fibers found in the muscle and increased the contractile speed in the absence of motor nerve alteration. Contrary to an earlier published report, implantation of cut motor neurons, which originally innervated fast muscle, into normal innervated soleus muscle produced no attributable effects on the histochemical properties or contractile responses of the muscle.

Significance to Biomedical Research and the Program of the Institute: Adaptive modification of muscular responses, both histochemical and physiological, to a changed functional requirement and particularly in the absence of nerve alteration suggests a possible information input to the muscle other than through the motor neurons. These experiments emphasize that many factors contribute to the characterization and regulation of skeletal muscle responses each of which must be accounted for in any complete description of contractile mechanisms.

Proposed Course of Project: I plan to continue another aspect of this project in which the relationships between isotonic and isometric contractile rates are investigated in muscles of various mammals commonly used in this laboratory.

Honors and Awards: None

Publications:

Guth, L. and Wells, J. B.: Physiological and histochemical properties of the soleus muscle after denervation of its antagonists. Exp. Neurol. 36: 463-471, 1972.

Serial No. NDS(I)-67 MN/AP 1416(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A Pharmacological and Toxicological Study of Neurotoxic Venoms

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B.S.
Ahmed Hassan Mohamed, Ph.D.

Cooperating Units: Department of Physiology, Ein Shams University, Cairo, Egypt

Man Years:

Total: 0.0
Professional: 0.0
Other: 0.0

Project Description:

Objectives: To obtain and investigate heretofore unavilable venomous substances. To determine the primary point of biologic activity and to explain the tissue and cellular mechanisms of these neuro-active substances. To fractionate the whole venoms into their active components.

Methods Employed: The above objectives require the use of a wide variety of whole animals, isolated tissue, and cellular research techniques involving toxicological, biochemical and biophysical techniques. Well known venoms are examined in order to establish a standard. A comparison of the standard with lesser known venoms facilitates the discovery of new properties of the various venoms with a minimum of search. Gel electrophoresis and column chromatography are used for venom fractionation.

Major Findings: Biological activity of venoms catagorized as neurotoxic has been found to progress with time an/ dose from initial paralysis of peripheral origin to cardiovascular toxicity, and eventually to CNS effects due indirectly to cardiovascular failure. Intra-arterially administered neurotoxic venoms damage the blood-brain barrier and permit entry of large dye molecules into the brain but intravenous administration of venoms is not followed by dye entry. This finding can be extrapolated to indicate that snake-bite victims would probably not have CNS damage from neurotoxin, a point of some therapeutic interest. Additional venoms are now available from specimens collected from many points throughout Africa during the past year.

Significance to Bio-Medical Research and the Program of the Institute: Past research on venoms and naturally occurring toxins has been pre-eminently successful in various aspects of biology.

The collection of venoms from poisonous species has made possible the scientific development of antivenins for the treatment of envenomation by poisonous animals. The research here is collaborative with and supportive of a program that is now designed to collect venoms from the entire African Continent (except for the Union of South Africa) and to develop polyvalent antivenom for therapeutic use.

Because of the accelerating toxicity to which modern man is exposed, the study of toxic and poisonous substances becomes of increasing importance. Only a few relatively common venoms have been investigated. Over 600 different species of poisonous snakes are believed to exist in the world. What wealth of biological tools are undiscovered in the venom of the lesser known species can only be surmized from the success of past research. The relationships among snake venom neurotoxins, enzymes, antivenins, toxicities, and therapeutics are almost completely unknown. The project, therefore, has excellent possibilities of increasing progress in medical research.

The recent finding that snake venoms contain substances that function as nerve growth factors make this project especially relevent to the program of the Institute. The most recent example of the value of this type of project to science is the use of bungarotoxin obtained from the krait snakes and made radioactive to identify and locate the acetylcholine receptor on skeletal muscle fibers.

Proposed Course of Project: The termination date of the project has been delayed for an additional year without additional funding to allow a further examination of the needs of the Institute for a continuation. If a continuing Institute interest is found, a further project extenstion can be sought.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70 MN/AP 1809(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Impaired Transfer of Information Between Nerve and Muscle

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B.S.

Cooperating Units: None

Man Years:

Total: 0.0
Professional: 0.0
Other: 0.0

Project Description:

Objectives: To find biochemical deficiencies that impair transfer of biological information between nerve and muscle.

Methods Employed: Suitable animals are made deficient in a component of a metabolic pathway suspected of being required for transfer of information between nerve and muscle. Efficiency of information transfer is studied in vivo. Restoration of function is determined by return of metabolic substance or a precursor to the animal during testing. Simultaneous comparisons are made between muscle types.

Major Findings: Last year it was stated that adult rats kept without dietary choline and allowed access to their total environment practice coprophagy, recycle choline and transfer information normally between nerve and muscle. Weanling rats denied choline became metabolically deficient as indicated by increased number of deaths, loss of hair, eye and kidney hemorrhage, cataracts, large fatty livers and slow growth rates. These severely choline deficient rats maintain a near-normal ability to transmit signals between nerve and muscle. This indicates that under severe choline depletion the nervous system can utilize or synthesize choline to a greater degree than certain other tissues or that motor nerves may have a low choline requirement. It is of general interest to the neurological sciences that animals with major defects in many organ systems because of lack of dietary choline have no apparent functional deficiency in the efferent motor nerves.

Weanling rats made methionine deficient by dietary controls fail to gain weight but do not exhibit the physiological symptoms of choline deficiency or a transmission deficit between nerve and muscle. Animals made both choline and methionine deficient likewise fail to develop a defect in transfer of information from nerve to muscle. These later findings can be interpreted to mean that alternate pathways can supply one-carbon fragments or methyl groups in sufficient quantity to synthesize sufficient choline and subsequently enough acetylcholine to maintain transmission of information between nerve and muscle. The supplying of methyl groups by synthesis through one-carbon metabolic pathways would function as a protective mechanism for the nervous system against dietary deficiency of either choline or methionine. The functioning of such a protective system would have heuristic implications for organism survival since the nervous system, the primary organ for integrative functions, would be preferentially protected from dietary insufficiency of a chemical necessary for normal function. This year the project has been relatively inactive because the Principal Investigator is on detail as the Assistant Director of the Intramural program.

Significance to Bio-Medical Research and the Program of the Institute: There is great need for new knowledge about the processes involved when muscle fails to function in neuromuscular disorders. There is incomplete information about the biochemical substances that maintain the functional and structural integrity of muscle in the normal state. Identification of metabolic chemicals that are necessary for transfer of information from nerve to muscle may aid in determining the pathogenesis of some of the neuromuscular disorders, a useful step in designing of therapeutic measures.

Proposed Course of Project: This project should be continued with an emphasis on identification of the biochemical substances that are necessary to maintain the functional integrity of skeletal muscle. The future of this project has not been defined since the Principal Investigator is on detail as the Assistant Director of the Intramural program.

Honors and Awards: None

Publications: None

# ANNUAL REPORT

July 1, 1972 through June 30, 1973
Surgical Neurology Branch, IR
National Institute of Neurological Diseases and Stroke

J. M. Van Buren, Chief

## UMMARY OF STUDIES IN SURGICAL NEUROLOGY:

he primary orientation of the Branch is to study the form, function and isease of the human nervous system as relevant to neurological surgery. ong-term projects deal with studies in the following categories: I. yskinesias, II. Epilepsy, III. Speech, Memory and Cognitive Function, IV. rauma of the CNS, V. Tumors and VI. Vascular Disease of the CNS. A pilot tudy is being made in Pain.

### . Dyskinesias (Stereotaxic Program)

Basic Studies:

A. Studies continue of simultaneous unit activity in the cortex and thalamus of patients undergoing thalamotomy. Some cortical cells were found to be active in relation to motor movement (tremor, voluntary flexion or extension of the opposite extremity). A few showed a relationship to the activity of thalamic neurones.

B. Study of the retrograde degeneration in the human dentate nucleus has followed preliminary quantitative evaluation of normal specimens. Pathological cases have ranged from complete lenticulo-thalamic destruction through focal surgical thalamic lesions to essentially complete destruction of the brachium conjunctivum. Preliminary estimates indicate that 30-50% of dentate efferents enter the human thalamus. No distinct topographical correlation has appeared to date.

Clinical Studies:

A. A formal review of clinical testing procedures for dyskinesia has been completed. This points up the scant concern with quantitation of motor function even in terms of simple function-time relationships and the lack of practical methods for producing quantitative data suitable for analysis of human motor pathophysiology as an engineering problem. The intuitive nature of most evaluations lead to false claims and misinformation on the treatment of varying diseases from dyskinesias to stroke and multiple sclerosis.

### I. Epilepsy (Surgical Epileptic Program)

Basic Studies:

A. There has been technical improvement in the equipment for fluorometric assay of NADH in exposed cerebral cortex in Man in cooperation with the EEG Branch. The new device incorporates TV imaging and taping of an entire craniotomy field. This method permits comparative NADH assay of any part of the field at a given moment and thus permits following the topographic distribution of activation of cortex following a stimulus of a given point (e.g. NADH reduction following stimulation was seen to spread longitudinally along the 2nd temporal gyrus and not vertically into the adjacent 1st and 3rd gyri). Real-time analysis is needed before this can be a useful clinical tool.

B. Primate anatomical studies of importance in the central propagation pathways of cortical seizure discharge have indicated (Nauta and cell degeneration) major connections between the anterior insula and the "arcuate nucleus" (Vc i pc) lying immediately anteroinferior to the centrum medianum.

Clinical Studies:

A. Recall of patients having undergone craniotomy for epilepsy 3 or more years ago continues with a total case file now numbering in excess of 300 cases. A case file questionnaire suitable for reduction to punch cards has gone through several revisions and is now reproduced in useable form. This includes evaluation of clinical, X-ray, EEG, surgical ECoG, medication, socioeconomic and emotional factors. Evaluation will start with the temporal lobe seizure series.

III. Speech, Memory and Cognitive Functions (Surgical Epileptic and Stereotaxic Program)

A. Amytal testing for lateralization of speech dominance has provided evidence as well of short-term memory defects. This memory defect was considered more profound in the right hemisphere speech dominant group. This group also did less well on standard test of I.Q. and memory. This may indicate that early left hemisphere damage and relocation of speech to the right hemisphere results in a lower quality intellectual system.

B. Analysis of results of cortical speech mapping showed the expected posterior temporal localization of speech deficits with stimulation. Detailed analysis, however, suggest that phonemic errors ("fan" for "flag") tended to occur in the superior temporal region while semantically related errors ("shoe" for "foot") tended to appear in the posterior temporal regions. The type of memory errors also differed with location. Anterograde (input) errors were seen with anterior stimulation whereas retrograde (retrieval) errors were recorded with posterior stimulation.

C. Recognition of material presented tachistoscopically was studied after temporal ablations. Right resections were associated with greater recognition defects than left temporal resections suggesting that the right side may have a more active role in extrastriate visual perception.

D. In comparing the effects of parietal and temporal lesions on several memory tests it appeared that the parietal lobe provided largely visual and the temporal lobe largely auditory memory. Short term memory was dependent upon its visual or auditory character, while long term memory seemed dependent upon a verbal or nonverbal categorization.

E. Stimulation of the human cingulum tended to disrupt short term memory. Stimulation on the right interferred with the recognition of complex visual patterns while on the left the difficulty lay in memorizing verbal items. In conjunction with the study of temporal lobe lesions, the cingulum appeared to afford "attention" needed for learning while the temporal lobe provided the memory "register".

F. Further study of the Frontostriatal Arrest Response and comparison with speech and intellectual defects associated with subcortical stimulation in other sites have provided further evidence that frontostriatal stimulation blocks a basic mechanism sustaining consciousness.

## IV. Trauma of the CNS (No In-House Clinical Program)

Basic Studies:

A. Regarding the mechanics of head injury, studies in the squirrel monkey have shown that pure translational acceleration of over 1000 G produce only focal brain lesions while equivalent rotational acceleration produces cerebral concussion as well as diffuse brain lesions. The trauma models provided by these experiments can now be used for testing preventive and therapeutic measures.

B. Investigation of intracranial pressure mechanisms has shown in monkeys that the Pressure-Volume (P-V) curve is extremely rate sensitive with vascular engorgement serving as the chief cause of the exponentially rising part of the curve. Thus at low rates of volume increase, the vasopressor response is not elicited. As a result the P-V curve retains a low bell shape providing respiratory factors remain normal.

Clinical Studies:

A. In 11 patients with subdural hematoma, abnormal CSF flow patterns with radionuclide cisternography were observed. Reduced or arrested flow appeared in the subarachnoid spaces of the convexity underlying the hematoma. This abnormality persists after evacuation of the lesion, at least in the early postsurgical period.

## V. Tumors of the CNS (Clinical Tumor Program)

Basic Studies:

A. Three glycoproteins have been extracted from glioma tissue in higher concentration than control brain tissue. These are now being characterized as possible tumor specific antigens.

B. Spinal CSF flow patterns have been studied in the monkey by radioisotope methods. Following ventricular injection flow occurs into the spinal canal and this effect is more marked after cisterna magna instillation. On the other hand, flow from the anterior basal cisterns is directed exclusively toward the brain convexity.

C. A study is being undertaken with view to the possibility that immunotherapy may complement or supplant chemotherapy in the treatment of malignant gliomas. The effect of intracerebral PPD after intradermal BCG injection is being evaluated in monkeys to evaluate the risk of nonspecific enhancement of cellular immune response in the brain.

Clinical Studies:

A. Diagnostic Methods:

1. Cisternography in over 10 patients with supratentorial hemispheric gliomas has shown failure of ascent of the isotope on the side of the tumor, presumably due to local compression of the subarachnoid space. Of interest was the finding that 3 of these cases had negative $^{99m}$Tc pertechnetate conventional brain scans.

2. Radioisotope angiography of the spinal cord (IV injection) has proved to be a simple and safe method successfully demonstrating AVMs and hemangioblastomas.

3. While initial trials of the Tomoscanner were unsuccessful, modifications (particularly reduction of the inclination of the scanning probe) have improved resolution. The Tomoscanner, however, has not yet provided a significant amount of information beyond that obtained by conventional brain scanning. Since the potential value of the method is considerable, the technological and clinical studies will continue.

4. Study of supine myelography is being undertaken both with clinical films and postmortem material to investigate the nature and significanc of filling defects. Normal defects in the dye column have proved difficult to evaluate and differentiate from lesions of the spinal cord.

5. Preliminary inspection of the new device for computerized axial tomography of X-ray absorption in the brain (EMI-scanner) indicated that it is the first practical noninvasive device providing a quantitative index of response to tumor therapy. Funding is being sought.

### B. Therapeutic Methods

1. Patients with malignant gliomas showed decreased cellular immunity to DNCB whereas patients with grade I and II gliomas responded as did the controls. This finding has given impetus to the study of possible immunotherapeutic methods of glioma treatment.

2. Controlled trial of chemotherapy of grade III or IV astrocytomas after conventional decompression and radiotherapy has been carried out. The agents have been CCNU (130 mg./sq. m. body surface, 6 doses, q. 6-8 wks.) and intratumoral 8-azoguanine 100 mg. (weekly for 6 doses, then monthly thereafter). In 10 control patients the average survival was 12 mos. while the treatment group averaged 27 mos. survival. The EMI-scanner would appear to offer a decisive factor of morphological control in this study.

## VI. Vascular Disease of the CNS (No In-House Clinical Program)

Basic Studies:

A. Microangiographic studies have been carried out in 88 human spinal cords from 10 wks. of gestation to childhood. The "hairpin" arrangement of the radiculo-medullary arteries is evident from the earliest specimen onward. In the late fetal and postnatal period the anterior spinal artery is tortuous and the "watershed" areas are less evident than in adults. Studies continue on segmental variations and changes related to age.

B. Experimental work (Rhesus) in spinal cord angiography are continuing on vascular changes associated with postradiation myelitis and postangiographic myelopathy.

C. In a primate "stroke" model consistent infarctions have been obtained after arterial ligation, and microangiograms have provided precise time-pathology relationships. The effect of $PaCO_2$ has been studied, and its influence upon local tissue $O_2$ levels has proved variable. Angiographic evaluation of various types of major intracerebral venous experimental occlusions is underway.

D. A feasibility study has been conducted to evaluate the application of nuclear magnetic resonance techniques to estimate regional blood flow in man. Two possible principles have appeared: 1) marking the carotid inflow, depolarizing a localized intracerebral volume then determine the loss of activity in the jugular outflow (under investigation by NHLI), and 2) a spin-echo technique whereby a spectrum of flow velocities for a given cerebral volume might be obtained. Since the procedure is noninvasive and infinitely repeatable, funding for further contract research is sought.

Clinical Studies:

A. Study continues of the spinal cord vasculature and the changes associated with AVM's, tumors, postradiation myelitis, traumatic lesions, and, recently, certain cases with thoracic intervertebral disc disease. In thoracic disc protrusions the procedure provides precise evaluation of the degree of impingement of the disc upon the cord and its vessels.

B. Three paraplegic complications of aortography have been successfully treated by spinal lavage with physiological saline. This is accompanied by a decrease of CSF iodine content.

Branch Orientation and Planning:

No change is contemplated in the general character of the patient disease categories under study or the emphasis upon the study of the human nervous system and its disease. Due to the popular interest in Acupuncture, a pilot study is being made of this technique as a possible approach to the more general problem of Pain. Adequate controls for a study of this kind will have to be established.

Special attention will be given to improving the Epilepsy program by incorporation of newer surgical therapy (H-field lesions, cerebellar stimulation) and the possibility of histochemical study of subcortical pathways of seizure spread. Of first priority is recruitment of a neurochemist to assist in this area and with the Glioma study. A cerebrovascular physiologist will join us in July 1974.

Training of a Branch member as an Electron Microscopist is underway. At lower priority, a physiologist or behavioral psychologist will be sought who could undertake evaluation of motor function both in the experimental model and the clinical situation. Spatial and investigational consolidation of the Surgical Neurology program remains a major goal.

Serial No. NDS(I) - 54 SN/OC 100(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Epileptogenic Mechanisms in the Brain of Man and Other Primates

Previous Serial Number: Same

Principal Investigators: J. M. Van Buren, M. D., A. K. Ommaya, F.R.C.S. and C. Ajmone-Marsan, M. D.

Other Investigators: P. Fedio, Ph.D. and D. Lewis, M. D.

Cooperating Units: N-EEG

Man Years

| | |
|---|---|
| Total: | 1.6 |
| Professional: | 1.2 |
| Other: | 0.4 |

Project Description:

Objectives:

1. To study causal mechanisms of epileptic seizures in man and other primates.

2. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.

3. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.

4. To make use of opportunities in diagnosis and therapy for the study of neurophysiological and neuropsychological problems.

Methods Employed:

1. Clinical neurological examination.

2. Special radiographic and other contrast examinations.

3. Electrographic, including electrocorticographic, examination.

4. Physiological and psychological techniques as indicated.

5. Histological and chemical examinations as required.

Major Findings:

1. The surgical material has provided brain tissue for special neurochemical studies which are reported elsewhere (Serial No. NDS(I) - 69 SN/CN 1655).

2. Stimulation of implanted depth electrodes at open craniotomy have provided material for study of speech, memory and cognitive functions which are reported elsewhere (Serial No. NDS(I) - 57 SN/CP 401(c) and NDS(I) - 68 SN/OC 1658(c)).

3. Study of the late follow-up results of temporal epileptic excisions has again suffered from lack of staffing. During the past year however, a detailed questionnaire of some 800 entries has been worked out in cooperation with the Office of Biometry, NINDS, and has gone through several revisions. The present form is thought suitable for the study and has been prepared in final form. A Visiting Fellow has become available for this work in the Spring of 1973 so that it is hoped to prepare the basic data for punch card tabulation by the Fall of 1973.

4. Continued use has been made of the fluorometric method of NADH determination during open craniotomy as the occasion has arisen. Further work on the basic aspects of this problem have been carried out in the Electroencephalography Branch.

An innovation in the past year has been the introduction of TV imaging (and taping) of the fluorometric image of the entire craniotomy field. The first human case using this technique was done in March 1973. This method permits comparative NADH assay of any part of the field and thus permits following the topographic distribution of activation of cortex following stimulus of a given focus. For example, in this first case loss of NADH following a stimulus passed distally along the second temporal gyrus but did not spread vertically to the first and third temporal gyri. At the moment this analysis is rather time consuming and so can not be done on a real time basis (as the ECoG) for guidance of the surgeon. The method, however, appears of much interest as a research tool with potential for practical clinical use. (Details of the method are given in Annual Report FY 1972.)

Significance to Bio-Medical Research and Program of the Institute: Since the fluorometric estimation of NADH provides direct, real time, evidence regarding the intracellular metabolism of normal and epileptic cortex, it must be considered as a technical breakthrough. The recent TV imaging provides a capability for large field scanning and offers a clinical tool of much theoretical importance.

In the past year Rasmussen has begun to publish the results of the Montreal series of follow-ups of surgical resection for focal epilepsy.

Although their series numbers some 1600 cases, it is apparent that some of the features considered important in our study have not been made available. Thus our series will be pursued as originally planned.

Proposed Course of Project:

Histological control of the NADH recording sites is being continued in human material with conventional stains, metallic and Golgi impregnations and Epon embedding. The final histological study awaits the addition of an Electron Microscopist. Preliminary samples have been taken for study of the biogenic amine pattern of the epileptogenic material in collaboration with the Laboratory of Clinical Science, NIMH.

It is hoped to add a Neurochemist to the Surgical Neurology Branch in FY 1974 who will assist with the epileptic project both in study of surgical material and the chronic primate epileptic model with "mirror" focus.

The study of the late results of cortical resection for epilepsy will proceed. In addition two newer surgical procedures which have been reported to have good initial results will be evaluated as the opportunity arises. These are the lesions of Forel's fields (H-campotomy) and intermittent chronic cerebellar cortical stimulation with implanted devices.

Honors and Awards: None

Publications: None

Serial No. NDS(I) - 55 SN/OC 200 (c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Involuntary Movements

Previous Serial Number: Same

Principal Investigators: J. M. Van Buren, M. D., Choh-luh Li, M. D. and P. Fedio, Ph.D.

Other Investigators: D. Sadowsky, B.S.

Cooperating Units: N-OB

Man Years
Total: 2.4
Professional: 2.1
Other: 0.3

Project Description:

Objectives: Neurological disease characterized by dyskinesias offers a twofold opportunity for research. The pathological aspects of the disease itself may be studied as well as the pathophysiology of the motor system. The second aspect is the unparalleled opportunities afforded for neurophysiological studies in man by stereotaxic surgery in the treatment of dyskinesias. Studies made directly of the disease itself have not received great emphasis in the present research since they require biochemical and pathological support.

Methods Employed: Surface and depth microelectrode recording of the human brain makes use of specially built high impedance pre-amplifiers, tape recording of signals and eventually averaging and cross correlation of cellular activity and other functions such as muscle activity and brain potential variations from large electrodes. Depth electrode stimulation employs a specially built electrode and a current monitored stimulator. The special apparatus used in quantitative psychological testing is described in Dr. Fedio's Project Report Serial No. NDS(I) - 63 SN/CP 1033(c).

Major Findings:

1. Following the long-term results of Parkinsonian surgery (ctied in Bibliography) a study of the testing method was made with view to improvement of the procedures. This work may be summarized as follows:

Cognitive evaluation of 47 Parkinson patients for a mean of 17 months following unilateral thalamotomy, using the Wechsler-Bellevue Adult Intelligence Scale and the Wechsler Memory Scale, showed mild but definite cognitive declines persisting into the second postoperative year. Left-side operated patients showed declines mainly in verbally related scores and right-side operated patients showed declines mainly in nonverbal performance scores. These left-right differences were not secondarily caused by differences in age, sex, or degree of disease severity. A lack of adequate control data in the current literature prevents relating these changes directly to the thalamus. The changes observed are not sufficient in themselves to warrant cessation of thalamotomy in the treatment of Parkinson's disease.

2. Study of the curious "Arrest Response" whereby a patient is rendered unconscious by stimulation of the frontostriatal region (Van Buren et al., 1966) has been expanded by evaluation of their response to the Naming and Memory Test of Fedio (Neuropsychology, SN) under these conditions. Other areas in which stimulation has been carried out with the same test and thus serve as control areas include the lateral and mesial temporal areas, parietal and thalamic areas and cingulum. Subcortical speech areas on stimulation may show increased latency of correct responses. Frontostriatal responses, if correct, are not delayed. The stimulus is often recalled later if output is blocked by stimulation of a subcortical speech area. Block from frontostriatal stimulation so far has permitted neither response to or memory of the stimulus. Furthermore, about 25% of the left frontostriatal cases of stimulation have shown production of either garbled speech or speech unrelated to the testing situation without subsequent memory of the production. In sum, frontostriatal stimulation appears to attack consciousness itself as judged by loss of response to and absence of memory of the stimulus.

Significance to Bio-Medical Research and the Program of the Institute: The use of a human subject who is cooperative and unsedated permits many tests of sensory and psychological function which are impossible even with the time consuming conditioning experiments applied in lower animals. The use of the human subject is therefore not a duplication of animal experimentation but an extension of this and, of course, studies of speech function may only be carried out in man.

Proposed Course of the Project:

Since 1963 we have sought the support needed to set up a laboratory for quantitative evaluation of human motor function. Interest has been cited by Medical Neurology, NINDS, Section on Experimental Therapeutics, NIMH, as well as Surgical Neurology, without resulting in practical implementation. The "Workshop on Control of Movement and Posture" held March 27-28, 1972 under the sponsorship of Professor Granit has pointed out the importance and practicality of studies of this type. Transient liaison has been achieved with the former Chief, Technical Developments, NINDS and the Laboratory of Neurophysiology, NIMH but the lack of a facility to unite patients and investigators has led to rapid deterioration of the relationship.

From a clinical point of view a facility of this type is crucial for evaluation of any type of treatment dealing with the human motor system. From a research point of view, studies of this type are in their infancy and the entire field is wide open. It is important that NINDS leadership recognize this important area of study so that the support needed can be set up.

Hnoors and Awards: None

Publications:

Van Buren, J. M., Li, C.-l., Shapiro, D. Y., Henderson, W. G. and Sadowsky, D. A.: A qualitative and quantitative evaluation of parkinsonians three to six years following thalamotomy. Confin. Neurol. In press.

Serial No. NDS(I) - 56 SN/OC 304(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effect of Lesions Upon the Function and Structure of the Human Central Nervous System

Previous Serial Number: Same

Principal Investigator: J. M. Van Buren, M.D.

Other Investigators: R. C. Borke, B.S., M. G. Levine, M.A., F. P. Wirth, M.D.

Cooperating Units: None

Man Years

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|---|---|
| Total: | 2.4 |
| Professional: | 0.3 |
| Other: | 2.1 |

Project Description:

Objective: This project is directed toward the study of basic neuroanatomy and neurophysiology in man, making use of pathological material and the opportunities for study afforded by the operative treatment of neurological disease.

Methods Employed:

Anatomical studies:

1. Serial sections of human and animal brains in celloidin for myelin and Nissl series.

2. Section and staining of primate brains with Nauta technique for demonstration of degenerating pathways.

Major Findings:

1. Insular-diencephalic connections have been studied in five rhesus Macaques with both retrograde and anterograde degeneration techniques. Animals were prepared for study with bilateral symmetrical resections of temporal operculum or fronto-parietal operculum or both with or without excision of underlying insular cortex. The resections were staged such that the older was suitable for evaluation of retrograde cell change and myelin

tract degeneration (cresyl violet and Loyez myelin stain) and the more recent for anterograde fiber degeneration (Nauta). From the cresyl violet-Loyez stained hemispheres evidence was obtained for sustaining fiber projections to the insula from n. ventralis caudalis parvocellularis. Nauta stains demonstrated projections from the insular cortex to n. ventralis caudalis, n. ventralis caudalis ventralis and n. ventralis caudalis parvocellularis. Connections were also demonstrated to n. centralis, n. parafascicularis n. dorsalis oralis and the intralaminar nucleus. These connections closely resemble those of overlying fronto-parietal operculum and suggest that insular cortex is an extension of sensory-motor cortex as indicated by physiologic data.

2. By means of retrograde degeneration techniques, a study has been undertaken to determine diencephalic relationships to the dentate nucleus of the cerebellum in man. This complements the work finished last year in chimpanzee relating to the anterograde projections from lesions of the superior cerebellar peduncles.

Study of normal human specimens indicate that the volume of the dentate nucleus ranges from 105-216 $mm^3$ with a mean of 161.3 $mm^3$. The neuronal diameter ranges from 15.4-20.4 micra with a mean of 18.1 micra and the mean total neuron population amounts to 438,000 neurons, ranging from 401,000 to 533,000 cells. Similar evaluation of 2 of the 5 cases with pertinent focal lesions has been completed. This has indicated that following secondary degeneration there is no decrease in the volume of the nucleus or significant decrease in the diameter of the remaining neurons. Following "up stream" lesions significant losses in total neuron population occur in man. These preliminary figures suggest that about 30-50% of dentate efferents enter the thalamus in man.

3. Although the intralaminar thalamic nuclei have been considered traditionally to be without cortical connection, distinct degeneration has been observed in man with cerebral lesions in excess of 9 mos. duration. In 9 cases all lesions resulting in such degeneration lay in the frontal lobe. On reviewing this material a topographical correlation was observed preserving the anteroposterior and mediolateral relationships of the frontal-intralaminar relationships. It was apparent that in order for the intralaminar nuclei to degenerate the lesions had to be of long duration and of relatively large size as compared with the lesions causing degeneration in "specific" thalamic nuclei. The degeneration in the intralaminar nuclei was also accompanied by degeneration in the nuclei adjacent to the internal intramedullary lamina. "Control" lesions without intralaminar degeneration were available in the medial and lateral aspects of the remaining lobes with the exception of the medial parietal region.

Proposed Course of Project:

Work is being undertaken to continue reconstruction studies of lesions of the basal ganglia, particularly with correlation with the clinical aspects of the material and the secondary degeneration in other nuclei of the brain stem and diencephalon. It is hoped that this material will give a better understanding to the operation of thalamotomy which is heavily burdened by empiricism.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-62 SN/OC 907(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Trauma to the Nervous System.

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, M.D., F.R.C.S., F.A.C.S.

Other Investigators: S. Nakatani, M.D., T. A. Gennarelli, M.D., L. Thibault, M.S., B. Bhussri, Ph.D., A. Luessenhop, M.D., E. Rosvold, Ph.D., P. Fedio, Ph.D., M. Ebert, M.D.

Cooperating Units: Division of Research Services, Biomedical Engineering and Instrumentation Branch, NIH; Departments of Anatomy and Surgery (Division of Neurosurgery), Georgetown University School of Medicine, Laboratory of Neuropsychology, NIMH, Laboratory of Clinical Sciences, NIMH.

Man Years

|  |  |
|---|---|
| Total: | 2.0 |
| Professional: | 1.0 |
| Other: | 1.0 |

Project Description:

Objectives:

1. To understand the mechanisms of mechanical trauma to the nervous system and its responses to grades of such trauma.
2. To design rational methods for protection, prevention, and treatment of trauma to the brain and spinal cord.

Methods:

1. Utilizing programmable head accelerating devices, sub-human primates are subjected to controlled head accelerations, separating the injurious effects of inertial loading and contact phenomena of impact.
2. Utilizing a programmable contusion device and set of micro-balloon probes, the effect of controlled brain distortions at varying sites in the rhesus monkey is being determined.

3. Utilizing an on-line PDP-12 computer, evoked potentials are being tested as in-vivo, on-line indices of primary injury severity and onset of secondary brain lesions.

4. Utilize biochemical and histochemical techniques to determine the role of biogenic amines in the sequelae of neural trauma. Currently primate models to sample CSF continuously from ventricular cisternal and lumbar zones for these studies are being developed.

5. Utilize mathematical and physical models to provide the theoretical basis for utilization of the experimental data in the development of tolerance curves predicting neural trauma onset in man.

6. Develop tests for correlating injury severity with risks for onset of post-traumatic sequelae, with special reference to post-traumatic amnesia and epilepsy.

7. Correlate human and sub-human primate data on lesion and stimulation effects on memory mechanisms with special reference to the reintegration of neural functions after trauma.

Major Findings:

1. It has been shown in the squirrel monkey that pure translational acceleration exceeding 1000 G produce only focal brain lesions but never cerebral concussion. Rotational acceleration at equivalent levels invariably produce cerebral concussion as well as diffuse lesions. These data are in full support of our earlier hypothesis for the role of rotational stresses in brain injury.

2. Primate head injury models for both focal and diffuse head injury are now available for testing of protective and therapeutic measures.

3. The somatosensory evoked potential has been shown to be a quantitatively accurate index of the onset and duration of traumatic unconsciousness in primates and a reliable predictor for onset of secondary brain lesions after trauma.

4. The Pressure-Volume curve of the intracranial content has been shown to be an extremely rate-sensitive phenomenon with vascular engorgement serving as the chief cause of the exponentially rising part of the curve. Thus at low rates of volume increase the vasopressor response is not elicted and the P-V curve is a low bell-shaped curve provided respiratory factors are kept normal.

5. Analysis of lesion-stimulation data in patients undergoing surgery for epilepsy and intractable pain support the right-nonverbal/left-verbal organization of memory functions and in combination with earlier work in sub-human primates have provided the basis for a new hypothesis for memory mechanisms in man.

Significance to Bio-medical Research and the Program of the Institute:

1. Hitherto there have been no reproducible models for head injury available for quantitative evaluation of new diagnostic and theraputic regimes. Our current work has established such models in sub-human primates and work on improving head injury management can now proceed. Analysis of post-traumatic sequelae can also be conducted in the rhesus monkey.

Proposed Course:

1. Validation of current and new models of head injury to develop a rational set of standards for diagnosis and treatment as well as prevention and protection against trauma to the head.

2. Develop long term studies of post-traumatic sequelae after controlled head injury in primates.

3. Evaluate the SER as an index of head injury severity in man, with special reference to the in-vivo diagnosis of primary and secondary effects of trauma and their relative contributions to the genesis of post-traumatic sequelae.

Honors and Awards:

1. Chairman, International Conference on Neurotraumatology, Buenos Aires, Argentina. 1972
2. Invited Speaker International Conference on the Biokinetics of Impacts, Amsterdam (Netherlands) 1973
3. Invited Speaker The Fifth International Congress of Neurological Surgery, Tokyo, Japan. 1973
4. Appointed Consultant Editor, Journal Sport Medicine.

Publications:

Gennarelli, T.A., Thibault, L., and Ommaya, A.K.: A comparison of linear and rotational acceleration in the production of cerebral concussion. Proceedings of the 15th Stapp Car Crash Conference 1971. New York Society of Automotive Engineers pp. 797-803.

Gennarelli, T.A., Thibault, L., and Ommaya, A.K.: Pathophysiologic Responses to Rotational and Translational Accelerations of the Head. Proceedings of 16th Stapp Car Crash Conference 1972. New York Society of Automotive Engineers pp. 296-308.

Gerner, P., Ommaya, A.K., and Fedio, P.: A Study Of Visual Memory: Verbal and Nonverbal Mechanisms in Patients with Unilateral Temporal Lobectomy. Intern. J. Neuroscience, 4: 231-238, 1972

Letcher, F. S., Corrao, P.G., and Ommaya, A. K.: Head Injury in the Chimpanzee: II Spontaneous and Evoked Epidural Potentials as Indices of Injury Severity. J. Neurosurg. (in press)

Nakatani, S., and Ommaya, A.K.: A Critical Rate of Cerebral Compression. In Intracranial Pressure Experimental and Clinical Aspects, Edited by Brock, M. and Dietz, H. Springer-Verlag 1972, pp. 144-148.

Ommaya, A.K., Corrao, P., and Letcher, F.: Head Injury In The Chimpanzee: I. Biodynamics of Traumatic Unconsciousness. J. Neurosurg., (in press)

Ommaya, A.K., and Fedio, P.: The Contribution of Cingulum and Hippocampal Structures of Memory Mechanisms in Man. *Confinia Neurologica*, 34: 398-411, 1972

Ommaya, A. K., Gennarelli, T., and Thibault, L.: Traumatic Unconsiousness: Mechanisms of Brain Injury in Violent Shaking of the Head. *Proc. Amer. Assoc. Neurol. Surgns.*, 1973 (in press)

Serial No. NDS(I)-62 SN/OC 913(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Single Cell Discharges from Various Nervous Structures and Their Functional Organization in Particular Reference to Somatosensory Activity in Man

Previous Serial Number: SAME

Principal Investigators: Choh-luh Li, M.D., Ph.D., John Van Buren, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.2 |
| Other: | 0.0 |

Project Description:

Objective: To study the activity of single nerve cells in the cerebral cortex and subcortical nuclei in relation to motor and sensory functions.

Methods Employed: Two micro-electrodes are placed, one in the motor cortex and the other in the thalamus of patients undergoing operative procedures. The other parameters, e.g. EEG, EMG, are also measured in response to motor movement or sensory stimulation.

Major Findings: Some cells in the motor cortex were found to be active in close relation to motor movement, such as Parkinson tremors, voluntary flexors or extension of the opposite extremity. A few showed suggestive evidence that the activity is dependent upon the activity of the thalamic neurons.

Significance to Biomedical Research and the Program of the Institute: The program will add to our knowledge of normal and abnormal functions of the motor system in man.

Proposed Course of Project: Multiple microelectrodes implanted in various structures of the striatum to record simultaneously the activity of the cells for long periods and to study the correlation of this activity and the specific motor movement of the patient will be a great leap forward in this line of research.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 SN/OC 1025(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Tumors of the Nervous System

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, M.D., F.R.C.S., F.A.C.S.

Other Investigators: Leland Albright, M.D. and W. Terry, M.D.

Cooperating Units: Immunology Branch, Division of Cancer Biology and Diagnosis, National Cancer Institute.

Man Years

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 1.0 |
| Other: | 1.0 |

Project Description:

Objectives:

1. To develop immunochemotherapeutic methods for control of inoperable brain tumors in man.
2. To develop diagnostic indices for the presence of such tumors and their response to therapy in man.

Methods Employed:

1. Patients with histologically verified glioblastoma multiforme and malignant astrocytomas (Grade III or IV) are selected. Adequate standard therapy (surgical decompression, radiation) and the extent of neurological deficit and intracranial mass anatomy is established clinically and neuroradiologically.
2. Cerebrospinal fluid reservoirs and intratumoral cysts are inserted to provide portals for infusion chemotherapy via the tumor cyst prosthesis.
3. A program of combined chemotherapy utilizing oral CCNU 130 mgm/sq. meter body surface and intratumoral 8-Azaguanine 100 mgm by infusion is started, the oral drug being given for six doses, one dose per 6-8 week period or until onset of liver or marrow disturbance. The intratumoral drug is given once a week for six weeks and then once a month for one year and once a month indefinitely after that.

4. Skin testing of patients with and without brain tumors using both common skin antigens (streptokenase-streptodornase) and DNCB to assess delayed hypersensitivity as an index of cellular immunity is being conducted.

5. Brain tissue removed from epileptic patients and glioma cases is subjected to the LIS technique of extraction for glycoproteins which are isolated electrophoretically on acrylamide gels. Characterization of tumor glycoproteins as tumorspecific antigens in animals is proceeding.

6. Intracerebral PPD given after intradermal BCG is being evaluated in the rhesus monkey for the risk of non-specific enhancement of cellular immune responses in the brain.

7. X-ray absorption in tissues is being tested as a new technique for noninvasive diagnosis and serial evaluation of tumors.

Major Findings:

1. The results of a controlled trial of our combined chemotherapy (after conventional therapy of surgery plus radiation) in 20 patients show that the mean survival time was increased from 12 months in the control group (10 patients receiving conventional therapy only) to 27 months in the group receiving additional combined chemotherapy (CCNU plus 8-Azaguanine). This result was significant for 50% improved survival at 2 years and for 25% improved survival at 3 years.

2. Skin testing data revealed that responses to common skin antigens in patients with gliomas were similar to those in patients with non-neoplastic disease. Responses to DNCB however showed significantly decreased cellular immunity in patients with Grade III and IV gliomas whereas Grade I and II glioma patients exhibited responses similar to patients without neoplasms.

3. Threee glycoproteins have been extracted from glioma tissue in much higher concentrations than found in adjacent non-neoplastic brain or in excised epileptic brain tissue. These glycoproteins are now being characterized as possible tumor specific antigens.

4. Preliminary results of personal inspection of the new British invention for computerized axial tomography (the EMI-scanner) indicate that the principle of quantitative measurement of x-ray absorption in brain tissues with this technique will provide the first non-invasive 'in-vivo' method for use as an index of tumor response to therapy.

Significance to Bio-medical Research and the Program of the Institute:

1. Successful immunologic rejection of a small tumor cell mass after initial surgical, radiotherapeutic and chemical reduction of the tumor volume will improve the presently inadequate treatment of glial tumors of the nervous system and hopefully extend clinical cures to patients with glioblastoma multiforme and other malignant gliomas. The development of our methods could conceivably lead also to preventive techniques of brain tumor management.

Proposed Course:

1. A new protocol comparing 3 modes of therapy, combined chemotherapy as above vs. immunotherapy alone vs. both combined is now being developed. This will be a prospective controlled study.

2. A phanthom head is being constructed and horizontal sections of a cadaver head (unstained) are being prepared for x-ray absorption studies and tissue calibration measurement directed at quantitative evaluation of the EMI-scanner technique as a diagnostic and prognostic tool in the management of brain tumors.

Honors and Awards: None

Publications:

Jones, A. E., Koslow, M., Johnston, G.S., and Ommaya, A.K.: Ga-Citrate Scintigraphy of Brain Tumors. Radiology, 105: 693-697, 1972

Ommaya, A.K., and Albright, L: Combined Chemotherapy and Immunotherapy for Malignant Gliomas. Proc. Amer. Assoc. Neurol. Surgns., 1973 (in press)

Ommaya, A.K.: Computerized Axial Tomography of the Head: The EMI-scanner, A New Device for Direct Examination of the Brain "In-Vivo". Surgical Neurology, 1973 (in press)

Serial No. NDS(I)-63 SN/NR 1047(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Isotope-Ventriculography and Isotope-Cisternography

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Eugene L. Timins, M.D.
Frank H. Allen, M.D.
Michael V. Green
Timothy Harrington, M.D.
Gerald S. Johnston, M.D.
A. Eric Jones, M.D.
Steven M. Larson, M.D.
Italo Rinaldi, M.D.
Sybil J. Swann

Cooperating Units: Department of Nuclear Medicine, Diagnostic Radioisotope Section, Clinical Center, NIH

The Hampton Roads Neurological Center, Newport News, Virginia

Man Years:

| | |
|---|---|
| Total: | .4 |
| Professional: | .4 |
| Others: | .0 |

Project Description:

Objectives: A gamma emitting isotope injected within the cerebrospinal fluid pathways will permit in subsequent head scans the pictorial outline of the ventricular system (isotope-ventriculography) and of the subarachnoid intracranial spaces (isotope-cisternography). Information about the anatomical status of the cerebrospinal fluid cavities, and, by multiple serial scans, of the normal and abnormal dynamics of the cerebrospinal fluid itself will be obtained.

Methods Employed: The radioisotope cisternography and ventriculography procedures are now well established.

Recently we have devoted particular attention to one aspect of the CSF flow, i.e. its descent to the spinal subarachnoid space. Experiments have been carried out in the Rhesus monkey after injection of radiopharmaceuticals within the ventricular system. The scintiphotographic data are appraised with the assistance of a computer. Digital analysis is performed using a small dedicated computer (HP 5407A Hewlett Packard Scintigraphic Data Analyzer), mated to the Anger camera. For this purpose the pin-hole collimator is positioned so that the entire lateral length of the cerebrospinal space is within the field of view of the Anger camera detector. Nine region of interest cursors are drawn: one over the cerebral convexity, and one to include the entire spinal subarachnoid space. Time-activity curves are obtained from each region of interest, simultaneously without moving the animal for the next three and one-half hours.

Major Findings: During the current fiscal year we have:

1) Gained additional experience with cisternography in cases of porencephaly.

2) Studied by radionuclide cisternography a group of 11 patients with subdural hematoma. In these cases decidedly abnormal patterns of flow were demonstrated. The most common finding was a localized area of decreased uptake, indicating arrested or reduced flow in the subarachnoid spaces of the cerebral convexity underlying the hematoma. Control postoperative cisternograms demonstrated that the localized area of reduced radionuclide flow persists after evacuation of the hematoma, at least in the early postsurgical period.

3) Initiated a study of supratentorial hemispheric gliomas by radioisotope cisternography. More than 10 patients with such type of neoplasia have been examined. In each instance a characteristic pattern of lack of ascent of the radiopharmaceutical on the tumoral side was noted. This is probably due to the encroachment on the subarachnoid space by the edematous hemisphere. Of particular interest are four cases of glioma showing the above described abnormal cisternographic pattern but with negative conventional Technetium 99m pertechnetate brain scanning (cerebral- and/or pneumoencephalography were, however, abnormal in three and borderline in one of these four patients).

4) Injected radiopharmaceuticals within the lateral ventricles of Rhesus monkey with the intent of studying the spinal CSF flow. A pattern of early spinal subarachnoid descent has been noted. This is followed first by equilibration, and later by decrease of spinal radioactivity as well as concomitant augmentation of cerebral convexity subarachnoid activity. When the radiopharmaceutical is introduced via the cisterna magna, the observed downward spinal pattern is even more marked, whereas an injection into the anterior basal cisterns is followed by a prevalent ascending direction of the tagged albumin toward the convexity of the brain.

5) Gained additional experience with radionuclide ventriculography in patients with intracerebral neoplasm, meningeal infection and low pressure hydrocephalus. Important applications were assessment of CSF shunts, determining distribution of intrathecally administered drugs, evaluating functional status of hydrocephalus and assessing the progression of neoplastic invasion of the ventricular system.

Significance to Bio-Medical Research and the Program of the Institute: Legions of authors are studying this remarkable fluid (CSF) which still remains uncomprehended since Cotugno first described it in 1764. In particular, we have now a diagnostic tool to gather information about the "terra incognita" which is represented by the basal and convexity subarachnoidal pathways.

Last year's investigations should have practical implication for the diagnosis and follow-up of such conditions as porencephaly, subdural hematoma, and hemispheric glioma. The observations on the spinal descent pattern of the CSF have heuristic significance in regard to a possible analysis of metabolites and drugs distribution through the CSF from the endocranial cavity to the spinal theca.

Proposed Course of Project: Further information about the normal and abnormal cerebrospinal fluid cavities, and the normal and pathologic flow of the CSF will be gathered by the techniques of isotope-cisternography and isotope-ventriculography.

Honors and Awards: Dr. Di Chiro has been chosen for the annual 1973 David M. Gould Lectureship, Johns Hopkins University. The main topic of this lecture will deal with radioisotope cisternography.

Publications: Di Chiro, G., Stein, S.C., and Harrington, T.: Spontaneous cerebrospinal fluid rhinorrhea in normal dogs. J. Neuropath. exp. Neurol. 31: 447-453, 1972.

Harbert, J.C., McCullough, D.C., Luessenhop, A.J., and Di Chiro, G. (Eds.): Cisternography and Hydrocephalus. A Symposium. Springfield, Illinois, Charles C. Thomas, 1972, 559 pp.

Rinaldi, I., Harris, W.O., Jr., and Di Chiro, G.: Radionuclide cisternography in subdural hematomas. Radiology 105: 597-602, 1972.

Di Chiro, G., Larson, S.M., Harrington, T., Johnston, G.S., Green, M.V., and Swann, S.J.: Descent of cerebrospinal fluid to spinal subarachnoid space. Acta radiol., Stockh., (Diagn.). (In press).

Harbert, J.C., McCullough, D.C., and Di Chiro, G.: Clinical applications of cisternography in pediatrics. Proceedings of the Symposium "Pediatric Nuclear Medicine," February, 1972. (In press).

Di Chiro, G.: Cisternography: Tribulations of a new diagnostic procedure. Hopkins Med. J. (In press).

Serial No. NDS(I)-65 SN/NR 1195(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Radiographic and Radioisotopic Angiography of the Spinal Cord

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Eugene L. Timins, M.D.
Louis Wener, M.D.
Mark M. Mishkin, M.D.
Jean R.L. Herdt, M.D.
Timothy Harrington, M.D.
Gerald S. Johnston, M.D.
A. Eric Jones, M.D.
Frank H. Allen, M.D.

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Department of Nuclear Medicine, Clinical Center, NIH

Department of Radiology, Cafritz Memorial Hospital, Washington, D.C.

Department of Radiology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

Medical Examiner's Office, City of Philadelphia, Department of Public Health, Philadelphia, Pennsylvania

Man Years:

| | |
|---|---|
| Total: | .6 |
| Professional: | .6 |
| Others: | .0 |

Project Description:

Objectives: The introduction of cerebral angiography (1927) has markedly increased our knowledge of the vascular pathology of the brain. The vascular pathology of the spinal cord, on the other hand, remains a largely unexplored area.

Since 1964, we have been carrying out angiographic studies of the spinal cord and developed this technique into a reliable diagnostic tool. Selective injection of the contrast medium has made the difference between an occasional demonstration, and the consistent visualization of the spinal cord vasculature.

The usefulness of selective arteriography in cases of spinal cord arteriovenous malformations is now well established. We are continuing to use this technique to: 1) Learn more about the pathophysiology of the spinal cord arteriovenous malformations so that a better treatment of these important and frequent lesions may be developed. 2) Evaluate how useful spinal cord angiography is in cases of spinal cord tumors. 3) Establish whether or not this technique can be of diagnostic value in the study of obstructive spinal cord vascular disease. 4) Assess the usefulness of this technique in intervertebral disc pathology. 5) Evaluate the diagnostic possibilities of this procedure in posttraumatic spinal cord injury with or without vertebral fractures. 6) Establish the value and limits of the newly introduced radioisotopic angiography of the spinal cord. 7) In a collateral, but strictly related study, we are trying to establish what types of emergency therapeutic means can be employed in an attempt to treat and cure, or at least minimize the effects, of the dreadful postangiographic cord complications. 8) Acquire new information regarding the fine vasculature of the human spinal cord, with particular emphasis on the intrinsic vessels (sulcal or central arteries and other perforating or penetrating branches). This goal is accomplished by postmortem microangiographic techniques in cadavers of all age groups. We have paid particular attention to cords in fetuses and in aged adults.

Methods Employed: Selective arteriograms with modern catheter techniques are carried out in patients in whom spinal cord vascular or tumoral lesions are suspected. The subtraction technique is used to better visualize the injected vessels. In addition, in the last fiscal year we have gained considerable experience with the direct radiographic magnification angiograms.

For the technique of radioisotope angiography of the spinal cord a bolus of 15 mCi of $^{99m}$Tc human serum albumin (1 to 2 ml) is injected in a left antecubital vein. Immediately afterwards, cinescintiphotographic or rapid flow Polaroid views of the various segments of the spine are obtained with an Anger scintillation camera. In the last fiscal year our scintiphotographic data have been significantly ameliorated by a computer assisted analysis and reconstruction of images, as well as by isometric contour computer display of the data.

For the studies of the vessels of the fetal and aged-adults' human spinal cords, we have used our previously developed microangiographic techniques.

Based on the observation, made elsewhere, that in two patients who died soon after aortography with spinal cord complications, the iodine content in the CSF was enormously increased, we are attempting an emergency therapeutic method consisting of flushing out the "iodine contaminated" CSF.

Major Findings: During the current fiscal year we have been able to further develop the technique and to augment our experience with selective arteriography of the spinal cord. In particular:

1) Additional experience has been gathered with the arteriovenous malformations and tumors (particularly hemangioblastomas), and obstructive vascular disease of the spinal cord.

2) Additional cases of arteriographic studies of the spinal cord in patients with posttraumatic para- and tetra-plegia have been carried out. Direct radiographic magnification has been particularly useful in these cases.

3) We have studied several patients with spinal cord pathology due to intervertebral disc disease. Particularly rewarding has been the information obtained in cases of herniation of the thoracic discs. Direct radiographic magnification has been especially useful in these cases. Only by spinal cord arteriography, we can reach a definite diagnosis of this condition together with an evaluation of the degree of impingement on the cord and its vessels by the protruding disc. This diagnosis is indispensable for a correct surgical therapy of this pathological condition: The exposure of the herniated thoracic disc should not be achieved through posterior laminectomy, but rather through a lateral retropleural or lateral transpleural approach.

4) We have continued our exploration of the angiographic findings in cases of suspected postradiation myelitis of the spinal cord.

5) A large number of patients have been studied with radioisotope angiography of the spinal cord. Striking demonstration of such lesions as arteriovenous malformations and hemangioblastomas are obtained by this simple, innocuous, and informative method. No diagnostic technique can match radioisotope angiography of the spinal cord in the screening and follow-up of patients with progressive myelopathy and suspected of harboring arteriovenous malformations or vascular tumors of the spinal cord.

6) The study on microangiography of the fetal human cord has been completed. The report dealt with a microangiographic study of 69 human fetal spinal cords, as well as 19 cords from infants and children. The youngest fetus was 10 weeks old. The characteristic "hairpin" arrangement of the thoracic and lumbar radiculo-medullary arteries is already evident in all our fetuses, including the smallest (10 weeks). The anterior spinal artery flows in a straight course in the younger to become tortuous in a number of older fetuses, term babies and younger children up to two years. The so-called "watershed areas" found in the anterior spinal artery in the adult are not clearly recognizable in the pre-birth cord.

The microangiographic evaluation of the aged human cord and a comparative appraisal of the intrinsic vasculature in the various segments (cervical, high- and mid-thoracic, and thoracolumbar) of the cord is underway.

7) In three patients who, in other hospital centers, developed paraplegia immediately after abdominal aortography, flushing out of the CSF and substitution with normal saline solution has been carried out, on an emergency basis, under our suggestion and guidance. This lavage has resulted in all three instances in a rapid amelioration of the paraplegia. The improvement has been concomitant with a decrease of the CSF iodine content. For comparison purposes we have been gathering CSF samples during the various stages of different angiographic procedures, particularly selective arteriography of the spinal cord. In patients without neurological complications the postangiographic raise of the iodine content in the CSF is minimal.

Significance to Bio-Medical Research and the Program of the Institute: Radiographic and radioisotopic angiography of the spinal cord are increasing our understanding of the large group of conditons in which vascular lesions of the cord represent the basic pathologic element.

Proposed Course of Project: We are "watching" for possible further technical developments of the technique of selective arteriography of the spinal cord. We have recently established the value of direct radiographic magnification, and we are considering initiating the use of angiotomography for a better visualization of the smaller vessels, possibly the intrinsic arteries and veins of the cord.

Improved X-ray vascular contrast media will also enhance the diagnostic possibilities of spinal cord angiography. We are following, very closely, the recent developments in the area of polimeric iodinated X-rays contrast media (dimers and trimers of various basic molecules), and we are planning to study some aspects of their neurotoxicity.

We will dedicate much of our attention to technical improvements in the newly introduced radioisotope angiography of the spinal cord. This method, which we are extensively using as a screening and follow-up procedure, could become a more definitive and informative diagnostic examination. By increasing our resolution through a computer-assisted reconstruction and enhancement of the images, we should be able to extract a lot of diagnostic information from this simple and innocuous technique.

Postmortem microangiography of the aged adults' cords should offer new insights on such conditions as obstructive vascular disease of the cord due to arteriosclerosis and cervical spondylosis, and possibly on degenerative and demyelinating cord diseases.

Honors and Awards: None

Publications: Di Chiro, G., and Fried, L.C.: Blood flow currents in spinal cord arteries. Neurology, Minneap. 21: 1088-1096, 1971.

Di Chiro, G., Jones, A.E., Johnston, G.S., Ommaya, A.K., and Koslow, M.: Radioisotope angiography of the spinal cord. J. nucl. Med. 13: 567-569, 1972.

Di Chiro, G.: Recent successes and failures in radiographic and radioisotopic angiography of the spinal cord. Brit. J. Radiol. 45: 553-560, 1972.

Stein, S.C., Ommaya, A.K., Doppman, J.L., and Di Chiro, G.: Arteriovenous malformation of the cauda equina with arterial supply from branches of the internal iliac arteries. Case report. J. Neurosurg. 36: 649-651, 1972.

Di Chiro, G., and Herdt, J.R.: Angiographic demonstration of spinal cord arterial occlusion in postradiation myelomalacia. Radiology 106: 317-319, 1973.

Di Chiro, G.: The development of spinal cord angiography. Acta radiol., Stockh. (In press).

Di Chiro, G., Harrington, T., and Fried, L.C.: Microangiography of human fetal spinal cord. Amer. J. Roentgenol. (In press).

Di Chiro, G., and Wener, L.: Angiography of the spinal cord: 1973 state of the art. J. Neurosurg. (In press).

Serial No. NDS(I)-66 SN/OC 1245(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: EEG Learning Correlates using Scalp and Intracranial Depth Electrodes.

Previous Serial Number: SAME

Principal Investigator: Paul Fedio, Ph.D. and William Sheriff, M.A.

Other Investigators: J. Van Buren, M.D., Ph.D.; A. Ommaya, F.R.C.S.

Cooperating Units: Laboratory of Psychology, NIMH; Section on Technical Development, NIMH

Man Years:
Total: .3
Professional: .2
Other: .1

Project Description:

Objectives: To identify cerebral mechanisms in man which mediate perception and the storage and retrieval of information.

Methods Employed: An auditory closure task consisting of discrete acoustic information was used, the stimuli being patterned to yield either meaningful or nonsense information. Electrographic (EEG) activity from scalp electrodes positioned over the left and right hemispheres was recorded. Included for study were patients who had undergone unilateral temporal lobe resections and patients with chronic, indwelling electrodes implanted in the thalamus or temporal lobe.

Major Findings: All electrographic test runs were conducted off line. The evoked potential data is currently being programmed for analysis by the PDP-12.

Significance to Biomedical Research and the Program of the Institute: Behavioral data available from epileptic patients following unilateral temporal lobectomy reveal significant perceptual and learning deficits which are related to the laterality of temporal surgery and to the verbal or nonverbal character of the material. The technique employed in this project affords a more precise method for outlining the network of cortical and subcortical structures in the human brain that guide behavior.

Proposed Course of the Project: To develop additional tasks and programs for EEG data analysis with wider application to patients with different neurologic disorders.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 SN/NR 1413(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Radioactive Scanning Tomography of the Brain

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Floro Miraldi, M.D., Ph.D.
Eugene T. Yon, Ph.D.
Gerald S. Johnston, M.D.

Cooperating Units: Department of Nuclear Medicine, Clinical Center, NIH

Department of Biomedical Engineering, and University Hospital, Case Western Reserve University, Cleveland, Ohio

Man Years:

| | |
|---|---|
| Total: | .1 |
| Professional: | .1 |
| Others: | .0 |

Project Description:

Objectives: Radioisotopic brain scanning is fast becoming one of the essential diagnostic methods to explore the brain. Its reliability in detection and localization of intracranial space-occupying lesions is high (about 80%) but still somewhat lower than that of other neuroradiologic procedures (cerebral angiography, pneumoencephalography). To improve the accuracy of brain scanning, we may essentially follow two research avenues: 1) better tracers; 2) improvement of the equipment. The improvement of the equipment is aimed at increasing the resolution without a substantial loss of sensitivity. This double goal will undoubtedly be reached if an effective and efficient method of layer body section (tomography) associated with scanning can be developed.

Methods Employed: A radioactive brain scan (obtained with a rectilinear scanner) or a scintiphoto (obtained with a fixed device such as a gamma scintillation camera) represents a complex record of signals (photons) originating from the endocranial, cranial and facial tissues. All these signals superimpose in a pattern in which it is often difficult to discriminate between the various anatomical elements. An improved analysis of the scintigraphic record of the head would be attained by tomography. We have been theoretically analyzing the various methods tried by other investigators to solve the problem of scanning tomography. These methods fall into four general categories: 1) focusing collimator systems; 2) systems using collimators inclined to the plane of interest; 3) scanning with collimators in the plane of interest; 4) time-of-flight positron systems.

We have performed a mathematic appraisal of the three fundamental parameters: Sensitivity, resolution, and efficiency.

We have proposed a new approach: The strong focusing multi-layered Tomoscanner. Judging from theoretical considerations, this new system holds promises to be, in many ways, advantageous compared to the ones already tested by other investigators.

Major Findings: The prototype scanner was brought in early 1972 from Cleveland to Bethesda and installed in the Department of Nuclear Medicine in the Clinical Center. Back in Cleveland this device had been tested with radioactive phantoms and found to be highly satisfactory. Our results with human patients, however, were disappointing. While a strong tomographic effect was confirmed, obscuring transport shadows, mostly annular in shape, originating from layers above and below the plane of interest in the patient's head, did destroy the information content of the scans. We soon realized that the inclination of the scanning probe (45 deg) was excessive. It was clear that modifications were necessary, and particularly that a decrease of about 15 deg of the probe angulation was indispensable. In the late spring of 1972 the Tomoscanner was shipped back to Cleveland where it is now located in the Department of Nuclear Medicine of the Case Western Reserve University Hospital.

Since the shipment of the Tomoscanner back to Cleveland, significant modifications have been carried out. In particular, the angulation of the detecting probe has been changed to 30 deg, which seems to represent a satisfactory compromise.

Preliminary experience with patients using the improved Tomoscanner has proven promising. About 30 cases suspected of harboring intracranial space occupying lesions and a few patients with liver pathology have been studied so far. The blurring of the activity outside the plane of interest is clearly accomplished. By multiple sequential cuts of the head a layer by layer analysis is possible. Three cases of brain tumors--a falx meningioma, a convexity glioma and a metastatic lesion--have been shown clearly in the tomographic cut in the plane of interest. In these three, as well as in other neurological patients, we have not, however, been helped with significant information beyond that obtained by conventional brain scanning. On the other hand, in cases of liver metastatic lesions, we have been able to recognize tumoral foci which had not been evident in the conventional liver scans.

For a definite assessment of the true capability of the Tomoscanner a large number of patients must be studied.

Significance to Bio-Medical Research and the Program of the Institute: A successful and simple method of scanning tomography would revolutionize the field of neuroradiology, and parenthetically other fields of radiology. Brain scanning and brain scintiphotography are in fact essentially innocuous (no mortality or morbidity), causing no discomfort to the patient, "less radiation giving" (if proper isotopes are used), and cheaper than other neuroradiologic procedures. Brain scanning and brain scintiphotography are also simple, quick procedures and may in fact, in most instances, be carried out on an ambulatory basis without admitting the patient to the hospital. Last but not least, interpreting a brain scan or a scintiphoto is much simpler and straightforward than reading a cerebral angiogram or a pneumoencephalogram. If, therefore, the resolution of brain scanning can be significantly improved by tomography, a complete change-over of the neuroradiology departments will occur. Brain scanning and brain scintiphotography would acquire further diagnostic importance for the detection of intracranial tumors, bleedings, and infarctions following strokes. Some of these lesions--especially the ones of the infiltrating type--which today escape detection by any of our diagnostic neuroradiologic methods, would then probably be "picked up." An increase, in absolute terms, of the detecting accuracy of the neuroradiologic methods, taken as a "whole" would then occur.

Proposed Course of Project: To attain the goal of studying a large number of neurologic and other patients by tomoscanning a simple playback system of the tape on which the data are digitalized and stored is indispensable. An indexer (locator system) is now being built, and this will allow us to perform a rapid reanalysis of the information accumulated on the tape. With the locator we will be able to proceed in a rapid fashion with the appraisal of the equipment's capabilities.

Particular attention will be devoted to the evaluation of "Tomoscanning" in clinical material in conjunction with conventional brain scanning, especially in patients with brain tumors and other endocranial space occupying lesions. The Tomoscanner will also be evaluated in association with the techniques of radioisotope cisternography and radioisotope ventriculography. A limited amount of work with tomographic scanning in experimental models (monkeys which have been subjected to experimental stroke and monkeys in which radioisotope ventriculography is performed) is also anticipated.

Honors and Awards: None

Publications: Miraldi, F., Yon, E.T., and Di Chiro, G.: Tomoscanner: Theory and Prototype. In: Freedman, G.S. (Ed.): Radionuclide Tomography. In press.

Di Chiro, G., Johnston, G.S., Miraldi, F., and Yon, E.T.: The Tomoscanner: Clinical Experience. In: Freedman, G.S. (Ed.) Radionuclide Tomography. In press.

Serial No. NDS(I)-67 SN/OC 1417(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Changes in Physiological Properties of Brain Tissue at Low Temperatures and in Other Pathological Conditions

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Joseph Fenstermacher, Ph.D., Barbro Johansson, M.D.

Cooperating Units: C-LCP

Man Years:

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|---|---|
| Total: | 0.0 |
| Professional: | 0.0 |
| Other: | 0.0 |

Project Description:

Objective: To determine the resistivity of nervous tissues under various neurosurgical conditions.

Methods Employed: Cats under Fluothane anesthesia, hypothermia, hypertension, etc. were used. In one experiment, a monkey was used who was subjected to electrical measurements for a period of two months. The method of measuring electrical resistance of the tissue was previously described (Li, *et al*. 1968). With this method, the results were applied to the Maxwell's Equation and the extracellular space of the tissue was calculated. The calculated extracellular space was then compared with the findings obtained by the concentration profiles of radioactively labeled compounds following subarachnoid perfusion. The brains of the animal after the experiment were fixed, sectioned and stained with Luxol Fast Blue-Nissl and Luxol Fast Blue-PAS stains for histological examination.

Major Findings: The extracellular space of the cat's cerebral cortex was found to be between 15 and 20% and was unchanged when the temperature of the cortex was decreased from 37 to 15° C. The rate of CSF formation was, however, found to be markedly reduced by hypothermia.

In another series of experiments, a sudden increase of systemic blood pressure by 90 mmHg was found to break down the blood brain barrier, With the blood brain barrier broken down, the activity of the nerve cells was found to be changed or inhibited.

The results from experiments on brain edema show changes in the level of consciousness which are still being subjected to analysis.

Significance to Biomedical Research and the Program of the Institute: Change in extracellular space may be a measure of extracellular or intracellular edema of the cells. The latter, in turn, is a measure of the metabolic response of the nerve cells to various pathological or physiological conditions. It is hoped that the method herewith described will be applied to neurosurgical patients as a measure of the progress or improvement of the neurological conditions.

Proposed Course of the Project: Further study along this line will be made when more supportive research personnel are available and when the previous collected data are published.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 SN/OC 1424(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Response Modulation by the Limbic System in Man: Neuropsychological and Physiological Changes with Amygdaloid and Cingulate Lesions

Previous Serial Number: SAME

Principal Investigators: Paul Fedio, Ph.D. and Ayub K. Ommaya, F.R.C.S.

Other Investigators: J. Van Buren, M.D., Ph.D.; T. Zahn, Ph.D. and W. Sheriff, M.A.

Cooperating Units: Laboratory of Psychology, NIMH and Section on Technical Development, NIMH

Man Years:

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|---|---|
| Total: | .3 |
| Professional: | .2 |
| Other: | .1 |

Project Description:

Objectives: To identify structural-functional interplay of brain mechanisms which regulate intellectual and emotional behavior in man.

Methods Employed: Therapeutic benefits from bilateral cingulomotomy in reducing 'appreciation of pain' are examined by standard psychophysiologic measures before and after surgery. Recognition of and immediate memory for verbal material and complex visual patterns were monitored during electrical stimulation of chronic electrodes implanted in the cingulum and other structures in the limbic system.

Major Findings: A transient but severe impairment in the ability to memorize verbal items followed left, but not right, cingulum stimulation. A reverse pattern was observed with right cingulum stimulation--the patient was unable to remember complex visual patterns that could not be easily decoded into verbal elements. Further inspection of the memory disorder showed that, while sparing perceptual processes, left and right cingulum stimulation may have upset the mechanisms which deposit information in short-term memory store. Coupled with observations made on epileptic patients following left or right temporal lobe resections, these studies support a dual memory system within the limbic brain. That is, the cingulum provides 'attention' or 'affective color' during learning, whereas the information to be learned is shuttled through the hippocampal complex and is laid down in a memory register.

Psychometric observations after bilateral cingulomotomy failed to reveal comparable deficits in memory and intellectual achievement. This apparent dissociation of stimulation-lesion effects in the presence of clinical improvement adds support to the view that the cingulum energizes the learning process. These structures in some fashion, activate the more distal, hippocampal complex to receive specific information and to hold events in a memory reserve.

Significance to Biomedical Research and the Program of the Institute: This project provides an opportunity to define precise behavioral and autonomic concomitants of limbic functions in man. The observations contribute to a better understanding of neuropsychologic mechanisms involved in the emotional appreciation of learning and memory, pain and stress.

Proposed Course of the Project: To continue the study and to extend the protocol to include patients subject to therapeutic lesions in the temporal lobe.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/OC 1526(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Epileptic Neurons and Their Recurrent Axon Collaterals

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objective: To study the electrical properties of the individual nerve cells in the cerebral cortex under normal and induced pathological conditions.

Methods Employed: Glass micropipette electrodes were inserted into the cells in the motor cortex of anesthetized cats. The electrical activity of the cells were recorded; and, thereafter, electrical current of increasing intensity was applied. The electrical resistance of the cell was derived from the current-voltage ratio and the time constant of the cell membrane and capacitance were calculated. This was carried out before and after the application of strychnine to the exposed cortex.

In another series of experiments repetitive electrical stimulation was applied to the cortex through a gross electrode adjacent to the recording micropipette electrode, or to the individual cells through the recording micropipette electrode.

Major Findings: Some of the electrical properties of cortical neurons have been reported previously. The preliminary observations suggest that they are different from neurons under the effect of strychnine. For instance, the resistivity of normal cortical neurons measures $(9.17 \pm 2.68) \times 10^6$ ohms while that of the epileptic neuron was found to be in the order of 5 to $6 \times 10^6$ ohms. Further, epileptiform activity of the cortex requires "synchronous" presynaptic bombardments and there is good evidence of negative feedback through the axon collaterals to the epileptic neurons.

Significance to Biomedical Research and the Program of the Institute: The electrical properties of the cell determine the cell's "natural" behavior as well as its response to the environment. If the electrical properties of the epileptic neurons are different it seems likely that any effective treatment will restore the normal electrical properties. Similarly, the electrical measurements may serve as a criterion of the effectiveness of the treatment of prognosis of the epileptic disorder.

Proposed Course of Project: Further investigations will be carried out to determine the electrical properties of nerve cells in response to various convulsants and anticonvulsants.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/OC 1527(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Physiological Mechanism of Motor Function in the Cat

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.3
Professional: 0.2
Other: 0.1

Project Description:

Objective: To study the functional organization of the pyramidal and extrapyramidal pathways.

Methods Employed: Intracellular and extracellular micropipette electrodes were used to record the activity of the nerve cells in the motor cortex of the cat and stimulation was applied to the basal nuclear structures. In the past year stimulation was applied primarily to the caudate nucleus and nucleus ventralis lateralis of the thalamus.

Major Findings: The discharges of cells in the motor cortex increase or decrease and the membrane potential increases or decreases in response to stimulation dependent upon the pre-stimulation activity of the cell and the duration of stimulation. The results of this experiment are in the process of analysis for publication.

Significance to Biomedical Research and the Program of the Institute: This study provides further information about the interaction of the pyramidal and extrapyramidal system at the central level. The present investigator is aware, however, that the regulatory effect of the extrapyramidal system on motor function may also be found in the cells of the motor cortex itself because only 4-5% of the cells in the motor cortex have descending axons in the medullary pyramid. The accumulated data may eventually shed some light on the understanding of motor function of dysfunction.

Proposed Course of Project: Experiments have been planned on unanesthetized monkeys for the further study of the motor system. These experiments will employ multiple microelectrodes to record simultaneously single cell activity from various nervous structures. The activity of the cells will be co-related with the movement of the monkey. Thereafter, electrolytic lesions in certain basal nuclei will be made and clinical evaluation and electrophysiological studies will be repeated. Therapeutic agents such as L-dopa, reserpine, etc. will then be given and similar clinical and physiological studies will be carried out.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/NR 1654(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Experimental Spinal Cord Angiography

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Eugene L. Timins, M.D.
Jean R.L. Herdt, M.D.
David A. Kumpe, M.D.
Jack Fein, M.D.
Giulio J. D'Angio, M.D.
Henry Aceto, Jr., M.D.

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Armed Forces Radiobiology Research Institute, Bethesda, Maryland

Department of Radiation Therapy, Memorial Sloan-Kettering Cancer Center, New York, New York

Space Radiation Effects Laboratory, College of William and Mary, N.A.S.A., Newport News, Virginia

Man Years:

| | |
|---|---|
| Total: | .3 |
| Professional: | .3 |
| Others: | .0 |

Project Description:

Objectives: The clinical value of the NIH developed technique of selective arteriography in the management of arteriovenous malformations and tumors (in particular hemangioblastomas) of the spinal cord is now well established.

In order to expand the clinical applications of arteriography of the spinal cord we are working with experimental angiographic and microangiographic models in primates.

Previously, we have concentrated our attention on the area of experimental obstructive vascular disease of the spinal cord in the Rhesus monkey. In the past fiscal year much of our experimental investigation has dealt with such iatrogenic pathological conditions as postradiation myelomalacia and post-angiographic myelopathy.

In the specific area of postradiation myelitis we are particularly interested in establishing whether the basic pathological lesion of this dreadful complication is primarily neurogenic or vascular.

Methods Employed: Various methods of spinal cord angiography (pressor amines aortography, external abdominal compression aortography, selective arteriography), as well as various microangiographic procedures, are used in monkeys subjected to different types of surgical occlusive procedures, as well as to external irradiation.

Major Findings: During the current fiscal year we have refined our angiographic and microangiographic methodology to include selective techniques.

In the area of experimental postradiation myelitis we have established the radiation protocol and carried out the actual cord irradiation in a large number of monkeys.

Significance to Biomedical Research and the Program of the Institute: The area of clinical spinal cord angiography is still relatively new, and experimental work is needed to clarify many aspects--optimal technique, diagnostic possibilities, dangers and avoidance of them--of this useful procedure.

Proposed Course of Project: We will concentrate our attention on experimental postradiation myelitis, causes and nature of postangiographic myelopathy and continue to work on certain aspects of occlusive disease of the spinal cord.

Honors and Awards: None

Publications: Di Chiro, G.: Directional, reversible currents of blood flow in spinal cord arteries. *T.-I.-T. J. Life Sci*. 2: 39-45, 1972.

Serial No. NDS(I)-68 SN/OC 1658(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Hemispheric Development and Specialization of Intellectual Functions

Previous Serial Number: SAME

Principal Investigators: Paul Fedio, Ph.D. and John Van Buren, M.D., Ph.D.

Other Investigators: A. Ommaya, F.R.C.S.; N. Butters, Ph.D.; I. Samuels, Ph.D. and L. Rosenthal, A.B.

Cooperating Units: Veterans Administration Hospital, Boston, Massachusetts

Man Years:
Total: .8
Professional: .6
Other: .2

Project Description:

Objectives:

1. Outline brain mechanisms which code information to be held for immediate use (short-term) or for later, delayed recall (long-term).

2. Investigate whether the human brain registers visual and auditory information in different cortical areas, and whether the verbal or nonverbal character of the information is vital to this neural process.

3. Compare the effects of brain lesions during infancy vs. adulthood on the development and recovery of intellectual abilities.

Methods Employed:

1. With epileptic patients being considered for therapeutic brain surgery (unilateral temporal lobectomy), lateralization of speech to the left or right hemisphere was determined by the intracarotid injection of Sodium Amytal (WADA Technique). Verbal competence in the ability to identify and to remember pictorial objects was continuously monitored before and after left and right intracarotid injections of Amytal to provide observations on baseline performance, dysphasia and recovery.

2. The laterality and general outline of cerebral zones instrumental in perception and immediate memory of verbal and nonverbal material were mapped by stimulation of the cortex during neurosurgical treatment of epileptic patients. Behavioral indices during stimulation were monitored on an object-naming task and a random-form discrimination test, each with a short-term memory command.

3. A profile of perceptual and intellectual deficits was plotted for neurosurgical patients with focal damage limited to the frontal, parietal or temporal lobes.

Major Findings:

1. Amytal irrigation of the hemisphere dominant for language produced dysphasia with slow recovery of basic linguistic skills and a longer-lasting deficit in immediate memory. Conversely, intracarotid injection of Sodium Amytal on the contralateral side failed to disrupt language skills and had little interference upon memory. The drug-related difficulties in response integration were most exaggerated in memory spheres. Even after the dysphasic condition was totally remitted, the patients showed prolonged impairment in short-term verbal memory demonstrated by an inability to hold and to recall the name of a single object that had been correctly identified 12 - 15 seconds earlier. The majority of patients showed the predicted left-hemisphere control of language, and a small number of patients revealed the reverse--right hemisphere regulation of language.

Several differences between the left brain and the right brain speech groups became apparent. Although amytal deactivation of the non-dominant hemisphere produced comparable effects for both groups, pharmacologic depression of the dominant side for the groups was markedly different. Specifically, the right speech group was more dysphasic, exhibited a more pronounced and longer-lasting dysphasia and a more pervasive memory disorder. Moreover, the right speech dominant patients performed more poorly than the left brain speech patients on tests of intelligence and memory. This would argue that, possibly owing to early widespread damage to the left brain, the readjustment of language to the intact, right hemisphere results in a less efficient or organized intellectual system.

2. In a separate project, the ability to identify objects and to remember their names was studied during electrical stimulation of the exposed surface of the human brain. This speech mapping procedure, that is, identifying by electrical stimulation those cortical areas of the brain that are essential for the preservation of language, was adopted from the classic works of Penfield and Roberts.

As expected, mistakes in naming familiar objects were evident only with stimulation within the posterior temporal-parietal zone (Wernicke's Area). Stimulation with comparable intensity levels of areas outside this primary region did not modify naming skills. A preliminary analysis of the types of aphasic errors (omission, perseveration and substitution) shows that phonemic errors (fan misidentified as flag) were produced by stimulation in the region of the superior temporal gyrus adjacent to the auditory cortex. In contrast, substitution errors conveying semantically related qualities, ('shoe' identified as "foot;" 'matches' as "lamp") were incurred during stimulation of the posterior temporal region. These data suggest that the sensory and association cortices in the human brain contribute to information processing beyond the basic sensory level. Specifically, the primary sensory areas may add basic or concrete dimensions to the output, while the association areas of the brain are responsible for the higher order or abstract properties.

In plotting the distribution of memory errors it was also obvious that the different types of errors produced were dependent upon the stimulating site. Interference during input or registration of information (anterograde losses) was observed when the anterior temporal cortex was electrically excited. Disturbances in output or retrieval (retrograde losses) were recorded when the stimulating current was applied to the posterior temporal region.

Thus, retrieval of previously established information and recently registered information may be served by a common mechanism located in the posterior temporo-parietal region. In contrast, the left anterior temporal cortex, coupled with medial structures (hippocampal complex especially), accomodates registration of information that is to be held for immediate use.

3. In a study by L. Rosenthal, processing of visual perceptual information by the left and right cortical temporal zones was evaluated with epileptic patients who had had unilateral temporal lobectomies several years previously. Recognition thresholds in the central and left and right visual fields were determined for words and random patterns projected tachistoscopically for a very brief duration.

Resection of the right temporal lobe was associated with an elevated recognition threshold for words and random patterns, appearing in each of the three visual fields (left, center, and right). The left temporal patients were not impaired and performed at a level comparable to normal control subjects. It seems likely that there is a hierarchy of perceptual processing, and that with a minimal amount of information the letters and random designs were processed as units of visual information per se. The data suggest that the right temporal lobe assumes a dominant role in extra-striate visual perception.

4. In collaboration with Drs. Butters and Samuels, Aphasia Unit, Boston VA Hospital, patients with parietal lesions (VA Hospital) or with temporal lobe lesions (NINDS) were compared on a wide range of memory tests. It appears tenable that short- and long-term memory systems store information on different bases: information entry into short-term memory is dependent on its visual or auditory character whereas long-term memory is categorized

according to verbal or nonverbal material. This research has suggested that visual information is memorized by mechanisms located in the parietal lobe, and auditory input, by the temporal lobe.

Significance to Biomedical Research and the Program of the Institute: The investigations advance understanding of the development and organization of structural-functional relationships in the human central nervous system. The information also attends to the impact of cerebral dysfunctioning in producing dysphasia, dyslexia and kindred language disorders.

Proposed Course of the Project: The visual and auditory tasks will be modified to further delineate learning processes, and immediate and long-term memory in patients with lateralized cortical and subcortical lesions. Parallel studies of interhemispheric relations will be made during deep brain stimulation and during cortical stimulation of patients in the neurosurgical operating suite.

Honors and Awards: None

Publications:

Fedio, P.: Language and the brain: An overview. *Proc. Am. Ling. Soc.*, (In press).

Serial No. NDS(I)-69 SN/NR 1791 (c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Angiography of Cerebral Venous Obstructive Disease

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro, M.D.
Mary K. Hammock, M.D.

Other Investigators: None

Cooperating Units: Experimental Surgery and Clinical Medicine Section, Veterinary Resources Branch, Division of Research Services, NIH

Man Years:
Total: .0
Professional: .0
Others: .0

Project Description:

Objectives: "Stroke" is one of the pathological conditions which recently has been a target attacked from many different sides. For instance, in the last ten years, a great amount of research has been dedicated to the angiographic evaluation of the cerebrovascular obstructive disease. The major emphasis, however, has been put on the arteriographic findings. A perusal of the literature clearly shows that only scattered observations have been made regarding the phlebographic changes. The neuropathological studies (Hahn, Loeb, Zülch), on the other hand, have pointed out the fact that the obstructive venous pathology, although not as frequent as its arterial counterpart, represents an important nosologic entity.

We intend to explore the angiographic changes occurring in the cerebral vessels after experimental obstruction of the various brain venous channels (veins and dural sinuses) in a primate experimental model. We are particularly interested in establishing, on the basis of these experiments, which venous occlusions can be well tolerated and which are, instead, incompatible with good function. We will try to appraise, again angiographically, what types of compensatory mechanisms take place after the venous ligations. Do new venous draining pathways open, and if so, which and how efficient are these reserve discharge routes? The angiographic work will be carried out in close connection with an analysis of the clinical status of the operated animals.

The knowledge acquired in our experimental models will be extended to human material.

Methods Employed: Catheter arteriographic studies in the monkey (Macaca mulatta) are performed before and after ligation of one or several of the large endocranial venous channels.

Together with the post-surgical angiographic evaluation, an analysis of the clinical condition of the monkeys is carried out. After various periods of time following the venous ligations, the animals are finally sacrificed in order to carry out microangiographic and gross and microscopic neuropathologic studies.

We are planning microangiographic studies of brains (supplied by the AFIP) of patients who have died of possible cerebral thrombophlebitis.

Major Findings: We have acquired a significant degree of experience in the evaluation of the monkey cerebral angiography. We have been impressed by the remarkable similarity of the major, and even medium size vessels (arteries, veins, dural sinuses) in the Rhesus monkey and man. We have good evidence, therefore, that the primate model will be applicable to the goals of our research project.

We have carried out in a group of Rhesus monkeys, ligation of the superior longitudinal sinus in its anterior, middle, or posterior third. Cerebral angiographic studies have been performed before and at various time intervals after the ligation. We are now in the process of evaluating our angiographic, surgical, gross and light microscopic anatomo-pathologic findings in the operated monkeys.

Significance to Bio-Medical Research and the Program of the Institute: This project can increase our understanding of the pathophysiology of the cerebrovascular occlusive disease. Possibly, we will be able to recognize and separate specific conditions (mainly due to venous drainage impairment) in the large group of pathologic entities which are classified under the heading of "stroke."

Proposed Course of Project: Due to other commitments of one of the investigators (Mary K. Hammock) the research activity on this project has been interrupted during the past year, but will be resumed very shortly when Dr. Hammock re-joins our group with a full-time position beginning May 1, 1973.

We intend to continue our experimental investigation in monkeys.

Later on we intend to apply the acquired experimental knowledge to the human clinical experience.

Honor and Awards: None

Publications: None

Serial No. NDS(I)-71 SN/NR 1866(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Cerebral Blood Flow in Normal and Infarcted Brain by Radiographic and Radioisotopic Methods

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Eugene L. Timins, M.D.
Timothy Harrington, M.D.
A. Eric Jones, M.D.
Michael J. Gelfand, M.D.
Sybil J. Swann

Cooperating Units: Department of Nuclear Medicine, Clinical Center, NIH

Laboratory of Applied Studies, Division of Computer Research and Technology, NIH

Man Years:

| | |
|---|---|
| Total: | .8 |
| Professional: | .8 |
| Others: | .0 |

Project Description:

Objectives: Although an enormous number of studies have been carried out on the cerebral blood flow (CBF), the actual behavior of the small cerebral vessels (arteries, capillaries, and veins) in physiologic and pathologic conditions in not yet clear. In fact, in recent years, new concepts--luxury perfusion, intracerebral steal, blue arteries and red veins, loss of autoregulation--have been introduced. These concepts have not brought about increased clarification, but rather additional confusion. We are convinced that morphological analysis of the vessels still remains a potential source of significant information in the area of normal and pathological CBF. We intend, therefore, to pursue the matter of extracting information regarding the circulatory conditions of the brain from radiographic observations, substantiated in selected instances by radioisotopic methods and possibly other diagnostic and research techniques.

Methods Employed: Radiographic and radioisotopic observations of the cerebral circulation represent the main tools to be used in this study.

An experimental stroke model in Rhesus monkeys has been developed. The middle cerebral artery is occluded at its origin through the superior orbital fissure after the removal of the eyeball. This technique (Garcia) offers various advantages. Particularly important is the fact that the produced infarction is consistent in its anatomical location (medial-inferior district of the ipsilateral basal ganglia) and its extent. Also, because no craniectomy is needed, subsequent studies with radioisotope scanning are easily interpretable.

After production of the stroke, monkeys are followed in their clinical status and by the following laboratory tests.

A) Radiographic angiography is carried out before and after surgery.

B) Radioisotopic carotid angiography is performed before surgery.

C) Sequential conventional brain scans (once a week) are performed after surgery.

D) Sequential (once a week) post-surgery radiosiotopic carotid angiography is carried out. The radioisotope angiography data are gathered with an Anger camera and stored on tape. Subsequently, the data on tape are analyzed by a computer-assisted program. Multiple cursors are drawn in the central part and at the periphery of the infarcted area. The purpose of this approach is to evaluate the centripetal and centrifugal blood flow in the infarcted area at various stages of development of the infarction. The data should be of assistance in the evaluation of the circulation parameters in the initiating, evolving, complete, and resolving stroke. The centripetal and centrifugal flow is evaluated second by second after the intracarotid injection of the radiopharmaceutical.

E) Acute polarographic deep electrodes are used in some of these animals for recording oxygen availability.

F) At various time intervals after the creation of the stroke, the animals are sacrificed by a "sudden death" technique and immediately afterwards perfused with a special micropaque solution for postmortem microangiographic studies.

G) The "sudden death" is accomplished under various attempts at modifying the pathophysiology of the CSF: hyperventilation, hypercarbia, anoxia, hyper- and hypo-tension, use of vasodilators.

H) In a few monkeys perfusion with the radioiodinated albumin macroaggregates for scanning appraisal of the infarcted hemisphere has also been carried out.

Major Findings: We are very pleased with the experimental stroke model which we have developed. The possibilities of investigation with this model are practically unlimited. We have started evaluating the many data available to us from the first group of infarcted monkeys.

1) The stroke model used gives a consistent area of infarction in deep cerebral layers with a minimal amount of brain exposure.

2) Microangiograms prepared in the manner described can fix at a point in time the dynamic changes taking place in the microvasculature.

3) The method allows us to duplicate in deeper structures some of the observations of others on the reaction of surface vessels.

4) We can demonstrate changes in vessel morphology following $PaCO_2$ changes both in normal and infarcted monkeys.

5) Both hypo and hypercapnia decrease the perfusion of zones of ischemia during the acute phase. Hypocapnia accelerates the rate of ischemia to a greater extent than hypercapnia.

6) In completed stroke at the five day interval, hyperventilation hypocapnia may increase perfusion of nonischemic portions of the brain as compared to normocapnic and hypercapnic animals.

7) By polarographic depth electrodes determination a reversal of the normal O2a response noted in the ischemic brain to either low or high $PaCO_2$ levels has been observed. This would suggest that attempts at manipulation of cerebral blood flow to ischemic or infarcted brain using hypo or hypercapnia would not be consistently successful due to the variable response obtained.

Significance to Bio-Medical Research and the Program of the Institute: We are confident that with our stroke model we will be able to contribute both in the basic area of CSF pathophysiology of the infarcted brain, as well as in some practical management of the patients with stroke. Perhaps we will shed some light on such fundamental questions as: a) if and when we should hyperventilate patients with stroke; b) when, if ever, induced hypercarbia may be useful in these patients; c) what is the optimal level at which the blood pressure should be maintained during the various stages of the stroke; d) if, when, and which vasodilators should be used.

Proposed Course of Project: This is a long-range research project. The evaluation of the cerebral blood flow both in experimental models and in clinical material is still in its infancy, but we are convinced that it will develop into a full discipline.

Soon a method will be available to us to evaluate the changes of the superficial vasculature of the normal and infarcted brain. This method (Yamamoto and Feindel) is a fluorescein angiography technique which permits the appraisal of dynamic changes of the pial and subpial hemispheric vasculature. This, together with the above described microangiographic technique, will enable us to compare the differential responses--to hyperventilation, hypercarbia, anoxia, hyper- and hypo-tension, use of pressor amines and vasodilators--of the superficial versus the deep cerebral vasculature.

We are hopeful that the computer analysis of our radiosiotope angiography data in an around the infarcted area will be informative. In particular the determination of the centripetal and centrifugal blood flow in the area of the lesion should enable us to draw some conclusion on the mechansisms of resolution of the infarction and perhaps a better understanding of the important phenomenon of the luxury perfusion.

Honors and Awards: None

Publications: Harrington, T., and Di Chiro, G.: Cerebral microangiography after hypocarbia and hypercarbia. Stroke 3: 553-559, 1972.

Harrington, T., Major, M., Ommaya, A.K., and Di Chiro, G.: Oxygen availability in ischemic brain following hypocarbia and hypercarbia. Stroke 3: 692-701, 1972.

Harrington, T., and Di Chiro, G.: Effect of hypocarbia and hypercarbia in experimental brain infarction. A microangiographic study in the monkey. Neurology, Minneap. 23: 294-299, 1973.

Serial No. NDS(I)-73 SN/NR 2008(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Supine Myelography

Previous Serial Number: None

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Eugene L. Timins, M.D.
David O. Davis, M.D.
H.E. Fillinger, M.D.

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Department of Radiology, George Washington University Medical Center, Washington, D.C.

Laboratory of Pathology, National Cancer Institute, NIH

Medical Examiner's Office, City of Philadelphia, Department of Public Health, Philadelphia, Pennsylvania

Man Years:

| | |
|---|---|
| Total: | .2 |
| Professional: | .2 |
| Others: | .0 |

Project Description:

Objectives: The overwhelming majority of non-hydrosoluble X-ray opaque contrast medium (Pantopaque) myelographies are carried out with the patient in the prone position. For various technical reasons the examination with the patient in supine position is not routinely performed. Through the years we have become more and more convinced that for an accurate appraisal of the spinal canal and its content supine myelography is indispensable. We have collected a large number of cases in which the pathologic lesion (arterio-venous malformation, tumor, arachnoidal cyst, Arnold-Chiari malformation) was demonstrated only or better in the supine than in the prone myelograms. We have, therefore, decided to look systematically at the matter of supine myelography. We are trying to learn the normal and abnormal radiographic anatomy recognizable in the supine myelograms, as well as to assess their relative value as compared to the prone roentgenograms.

Methods Employed: Three investigation routes are being followed:

1) Collection of as many cases as possible of examinations in which both prone and supine myelograms have been carried out. We make a point that in each myelographic procedure which we supervise, both prone and supine films are taken. In this respect, we use comparatively large amounts of X-ray contrast medium (from a minimum of 12 cc to 24 cc and more).

2) Prone and supine postmortem myelograms are carried out using total substitution of the spinal CSF with X-ray contrast medium (up to 100 cc of Pantopaque are employed for this purpose).

3) In postmortem material we are analyzing, by naked eye and under the surgical microscope, the normal anatomical formations located in the posterior part of the spinal subarachnoid space. These formations are responsible for the various, peculiar, and still largely unexplained, negative (filling defects) and positive (contrast medium pools) images which are so often encountered in the supine myelograms. We are concentrating our attention on such meningeal formations located behind the spinal cord, as the septum posticum, other arachnoidal septa, and subarachnoidal pockets (diverticula).

Major Findings: We are in the process of analyzing our clinical and postmortem material. From preliminary observations it looks as if we will be able to achieve a mapping of the normal and pathological radiographic anatomy of the supine myelograms.

Significance to Biomedical Research and the Program of the Institute: An accurate appraisal and an exact understanding of the radiographic anatomy of the posterior part of the spinal canal, acquired through the consistent and systematic use of supine myelography, will undoubtedly improve the diagnostic mertis of myelography.

Proposed Course of Project: After gaining a clear comprehension of the radio-anatomical detail of the posterior part of the spinal canal in the normal supine myelograms, we will proceed to assess the value and limits of this technical modification for exploring the intraspinal pathology.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 SN/OC 2009(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neurological Basis of Acupuncture-Analgesia

Previous Serial No.: NONE

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Herbert Lansdell, Ph.D.; Maxim Koslow, M.D., Anthony Bak and Dolores Blessing, R.N.

Cooperating Units: Laboratory of Neurophysiology, NINDS and Veterinary Resources Branch, Division of Research Services

Man Years
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objective: To study the basic mechanisms and the efficacy of the so-called "acupuncture-analgesia."

Methods Employed: Actual experimental procedure has not started, and various necessary preparations are in process. It is planned that a group of healthy adults will serve as volunteers from whom physiological measurements of the somatosensory and autonomic nervous functions and blood chemistry will be made. The volunteers will then be subject to acupuncture which is supposed to reduce or eliminate pain sensation. Thereafter, similar physiological measurements and blood studies will be carried out.

Major Findings: Not yet obtained.

Significance to Biomedical Research and the Program of the Institute: Acupuncture-anesthesia or acupuncture-analgesia has become a subject of wide interest. As a result, a great deal of intellectual manpower and money of NIH have been used, not to mention the fact that untold amounts of money have been spent by the common people in the so-called "Acupuncture Centers" across the country. It is the responsibility of the medical scientists in this Institute to make a statement substantiated by scientific data as to whether acupuncture-analgesia is a form of placebo, autogenic suggestion (or hypnotism) or it is genuinely effective with a sound neurological basis.

Proposed Course of Project: If "Acupuncture-analgesia" is proven to be a form of hypnosis, the project should be terminated. If it is proven to have neurological basis, it should be continued with greater efforts and enthusiasm.

Honors and Awards: None

Publications:

Li, C.-l.: Neurological basis of pain and its possible relationship to to acupuncture-analgesia. *Am. J. Chinese Med.* 1: 61-72, 1973.

Serial No. NDS(I)-73 SN/OC 2010(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

roject Title: Mechanisms of Pain

revious Serial Number: NONE

rincipal Investigator: Choh-luh Li, M.D., Ph.D.

ther Investigators: Maxim Koslow, M.D. and Anthony Bak

ooperating Units: Laboratory of Neurophysiology, NINDS

an Years:
Total: 0.8
Professional: 0.6
Other: 0.2

roject Description:

Objective: To study the physiological mechanisms of pain and changes pain threshold by other sensory inputs.

Methods Employed: As the C-fibers of the peripheral nerve are primarily r mediating pain sensation, the C-fiber responses to electrical stimulation e chosen as an indication of pain sensation. The saphenous nerve of the cat exposed and bathed in liquid paraffin at a predetermined temperature and is unted on a specially-designed electrode assembly. Electrical pulses of rious intensity and duration are applied so that maximal responses of the -fibers are recorded. A great effort has been made to study these responses the peripheral level so that a better understanding of these responses will gained when they were recorded from higher levels of the central nervous stem. At the higher levels, the interaction of the C-fiber responses and -fiber responses are studied. In addition, recording with intracellular and tracellular microelectrodes at these levels will be investigated; thus, the sic mechanisms of pain sensation, which may be either reduced by an increase the threshold of the C-fiber responses or eliminated by a blockage of the fiber responses, can be better understood.

Major Findings: The first part of this research program, i.e., to study e C-fiber responses at a peripheral level, is near completion. It is clear at although the conduction velocity of the C-fiber varies with the mperature, the strength-duration curves obtained between 27 and 37° C show significant difference. Further, the repetitive discharges, which occur following the A-fiber response and preceeding the C-fiber response are found to be an efferent event as a result of the repetitive discharges from the internuncial cells of the spinal cord, instead of A-fiber after-discharges as suggested by most physiologists.

Significance to Biomedical Research and the Program of the Institute: Pain is one of the major defense mechanisms in the body. It is no wonder that more than 70% of all patients complain of pain before they resort to medical advice. Yet, despite many theories on pain, the underlying mechanisms of pain remain little understood. The better we understand the underlying mechanisms of the symptom, the better we are in a position to control it. Since pain sensation is a function of the nervous system, it may be about time for the Institute of Neurological Diseases to investigate the mechanisms and management of pain sensation.

Proposed Course of Project: To continue studying the mechanisms and management of pain.

Honors and Awards: None

Publications: None

# ANNUAL REPORT
July 1, 1972 through June 30, 1973
Branch of Electroencephalography and Clinical Neurophysiology
Intramural Research
National Institute of Neurological Diseases and Stroke

Cosimo Ajmone Marsan, M.D., Chief

## Summary of Program Activity

### . Clinical-Diagnostic Service

This Branch has continued to provide such service for all the patients in the Clinical Center (both in- and out-patients) referred for EEG examination, either as part of their routine diagnostic work-up or as part of investigative projects originating outside the Branch (or Institute). This form of activity represents a considerable portion of the over-all activity of the Branch, monopolizing a total of over 5 man-years (0.70 professional and 4.65 technical-secretarial).

From the time of preparation of the last report (April 13, 1972) to that of the present report (April 13, 1973) a total of 1,303 EEG tracings have been obtained and interpreted in our Laboratory, on the basis of corresponding referrals from the various Institutes, and specifically:

| INSTITUTE | NO. | % |
|---|---|---|
| NINDS (OP - 376) | 863 | 66.3 |
| NCI | 164 | 12.5 |
| NIMH | 136 | 10.5 |
| NHI | 72 | 5.5 |
| NIAMD | 32 | 2.5 |
| NIAID | 26 | 2.0 |
| NIDR/ehs | 10 | 0.7 |
| | 1,303 | 100.0 |

The largest number of referrals have continued or originate from NINDS (and, predominantly, from the Branch of Surgical Neurology) while all other Institutes combined have accounted for one third of the referrals. The above figures include 28 tracings obtained from patients with chronically implanted electrodes. In addition, electrocorticograms were performed on 12 patients in the course of surgical exposure of the brain for the treatment of seizure disorders, in cooperation with the Branch of Surgical Neurology, NINDS.

2. Research Activity

Of the 19 projects included in the last Annual Report, 9 had been completed and 5 additional papers related to such projects have been published in the past year. Seven projects have been discontinued or terminated, mainly because of the departure of the principal investigator. Three projects have been continued through the current fiscal period and are included in this report together with four new projects.

Three of these investigations deal with the study of brain energy metabolism in both animals and man under various experimental conditions or pathophysiological states. These studies utilize the fluorometric monitoring of the changes in the ratio of oxidized to reduced nicotine adenine dinucleotide. In previously completed experiments in which such changes were monitored in the hippocampus, simultaneously with the changes in the local electrical activity in close proximity to an artificially induced epileptogenic process, interesting correlations had been found between these two parameters, especially during the development and course of ictal episodes. In one of the current projects, these studies have been continued and extended to investigate the effects of various concentrations of $K^+$ in both normal and "epileptic" (i.e., penicillin-treated) hippocampus. Significant decreases in NADH (i.e., increases in oxidative metabolism) were consistently obtained during the application of $K^+$ and these changes appeared to be related to its concentration; with 20-30 meq/lit $K^+$ concentrations, electrographic evidence of seizure activity was often present. Topical application of penicillin on this same structure is a well known method to induce a local epileptogenic process. In general, however, such process manifests itself electrographically with recurrent brief paroxysmal discharges of the inter-ictal type, while fully developed, long lasting episodes of ictal activity are exceptionally observed in the absence of repetitive electrical stimulation. In the current experiments it was found that certain $K^+$ concentrations have a marked 'activating' effect upon this epileptogenic process, being capable of inducing consistently organized seizure episodes; indeed, with critical $K^+$ concentrations, such episodes tend to become very frequent, the post-ictal phase of depression being progressively reduced until a "status-like" condition is obtained. In another of these three projects a new display method has been developed in cooperation with the Television Section of the Biomedical Engineering Branch, DRS. This method permits a very accurate topographical study of the

NADH changes. Such changes occurring in the superficial cortical layers are still monitored fluorometrically but with the use of a television camera and image intensifier. Both the resulting image of the exposed cortical field and the simultaneously recorded electrical activity are stored on video tape. After the experiment, the image is displayed on a TV screen by videotape playback. Changes in fluorescence intensity in any desired area of the image can be measured continuously using a gate generator and electronic densitometer. Thus, a rectangular area of any desired size can be selected and focused on any desired region to monitor its fluorescence intensity. The latter and its variations in time are displayed on paper together with the corresponding changes in electrical activity. By means of this method the entire area of exposed cortex can be sampled sequentially with repeated videotape playbacks and the changes of oxidative metabolism in different cortical areas analyzed in relation to the corresponding electrographic phenomena. This and the previous method are also currently applied to similar studies in the human cortex. The former should prove of particular usefulness in this application where time is at premium and the investigations cannot be extended for long periods as in the case of the experimental animal. The preliminary results of this study are promising but the limited number and the complexity of the cases from which they have been derived up to this time do not allow any definite conclusion. This project is expected to continue throughout the coming fiscal period.

One of the new projects is also in the field of experimental epilepsy but deals primarily with the disruption of normal functions in cortical neurons which participate in the epileptogenic process. In a previous project (described in one of the preceding Annual Reports) the effects of the paroxysmal 'bombardment' from epileptic neurons upon the function of normal cells located at some distance from the primary focus had been investigated. In the present project, the study deals with neurons of the visual cortex and, specifically, with the alterations in their firing patterns and receptive field properties which appear in the course of the development of an epileptogenic process and after its establishment in these same neuronal elements. The technique includes extracellular recording and local micro-iontophoresis of an epileptogenic agent in close proximity to the neuron under analysis, after determination of its receptive field properties. Specific results are described in the individual project description. These are still incomplete and this investigation will be continued through the next fiscal period.

The cellular mechanism which are possibly subserving the development of an epileptogenic process are investigated in another project. This study uses the cerebellum as an experimental model. This is one of the few brain structures which appears refractory to the action of all the most commonly used epileptogenic agents, either topically applied or systemically administered. A micro-electrode investigation of cerebellar neurons and of their membrane potential changes and firing pattern after topical application of several epileptogenic agents together with the knowledge of their characteristic synaptic circuitry should indirectly provide some information on the basic pathophysiological substrate of epileptogenesis in other CNS structures. Technical problems had to be overcome in this study and only preliminary findings are avilalbe at the time this Report is being prepared. It is anticipated that this project will continue for another year. In the clinical field the major project is a continuation of that initiated in the previous fiscal period and carried out in close collaboration with the Branch of Surgical Neurology, NINDS. The project deals with a long range follow-up study of the results of surgical treatment of patients with seizure disorders. Specifically, it deals with the effect of cortical excision on seizure frequency and pattern and with correlative studies of pre- and post-surgery EEGs and of electrocorticographic findings. The data on some of the several hundred patients on which this study is based have been collected and tabulated, and the conclusive findings will only become available after the entire series of cases has been processed and the data analyzed. The part of this project dealing with the technique, indication, limitation and interpretation of electrocorticography has been completed and this material forms the subject of a specialized monograph which is currently in press, as part of the Handbook of Electroencephalography and Clinical Neurophysiology. The results of the investigations which are still in course should prove of great practical interest not only for providing a much needed assessment of the usefulness of surgical treatment in focal epilepsy but also to point out the criteria for selection of those cases which are most likely to benefit from such therapy and, implicitly, for deciding against surgery in those types of seizure disorders for which a cortical excision is unlikely to yield beneficial results. At the present time there is still great uncertainty as to the diagnostic and prognostic significance of many electrographic (and clinical) data and the project in question should contribute useful information for, and clarification of these important aspects in the management of epileptic patients. Another clinical project also deals with seizure disorders and, specifically, with those relatively uncommon forms of seizures which originate from epileptogenic lesions located within the occipital cortex. On the basis of the electrographic findings, all the epileptic patients with evidence of occipital involvement have been selected and the clinical pattern(s) of their seizures analyzed and correlated to the specific EEG localization and lateralization. In a project completed in the previous fiscal period, a similar study had been carried out for patients with a fronto-rolandic-parietal localization of their epileptogenic process. The results of that study (currently in press) had provided a number of interesting data on the localizing and lateralizing value of many signs and symptoms in the ictal episodes of such patients. It is expected that the new, analogous study of patients with seizures of occipital origin, will contribute equally useful information of significance in the differential diagnosis and topographical classification of epileptogenic lesions.

3. Other Activity

The chief of the Branch is actively involved in editorial duties in several specialized journals and is currently co-editor of the Handbook of Electroencephalography and Clinical Neurophysiology consisting of about 30 volumes. As President of the International Federation of Societies for Electroencephalography and Clinical Neurophysiology, he is responsible for the organization and the scientific program of the International Congress to be held in Marseille (France), September 1973.

4. Programs considered for the near future.

The projects which are still pending should be completed in the coming fiscal year or in the following one. The main field of interest and investigations of this Branch remains focused on the various aspects (both basic and clinical) of seizure disorders and no major changes are anticipated in the immediate future.

Serial No. NDS(I)-72 EEG/CN 1939a
1. Electroencephalography and
Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Fluorometric monitoring of intracellular redox changes in the intact cat brain.

Previous Serial Number: Same (note modification in authorship)

Prinicipal Investigator: D. V. Lewis, M.D.

Other Investigators: M. J. O'Connor, M.D.

Cooperating Units: Division of Neurosurgery, Hospital of University of Pennsylvania

Man Years:

| | |
|---|---|
| Total: | 0.40 |
| Professional: | 0.25 (DVL) |
| Other: | 0.15 |

Project Description:

Objectives: to study energy metabolism in the normal and penicillin-treated cat hippocampus and the effect of $K^+$ and electrical stimulation on this metabolic activity.

Methods employed: Intracellular redox changes as indicated by changes in the ratio of oxidized to reduced nicotine adenine dinucleotide (NAD) are monitored fluorometrically in vivo in the superficial 1 mm of the exposed hippocampus. Electrical activity in this same area is monitored with surface electrodes. The hippocampus is continuously bathed in Elliott's B solution which is dripped onto the surface. Penicillin and/or $K^+$ are added to this solution and their effects on electrical and metabolic activity are recorded. The effect and interaction of electrical stimulation of the same region are also studied.

Major findings: In addition to previous findings, different levels of $K^+$ in the bathing of solution (Elliott's B) have been found to affect the frequency of ictal episodes as well as the duration of postictal depression in penicillin foci. Recurrent seizures have been elicited in a number of animals at and exceeding 12 meq/L of $K^+$; very similar interseizure intervals, seizure durations and postictal depression were seen at each level of $K^+$. Postictal silence was lost at high $K^+$ concentrations, so that a "status-like" condition would develop. Patterns of NADH changes were reproducible and varied slightly between animals and showed characteristic changes with each $K^+$ level. In addition, in the nonpenicillin-treated hippocampus higher concentrations of $K^+$, produced increases in oxidative metabolism which reached a maximum at 20 meq/lit. $K^+$, a concentration which was often associated with electrical seizure activity as well.

Significance to Bio-medical Research and the Program of the Institute: These studies show definite relationships between $K^+$ level, oxidative metabolism, and epileptiform activity. Further investigation could provide clues to the mechanism of seizure onset.

Proposed Course of the Project: A portion of this project has been completed and a paper is currently in press. The investigation of the metabolic events accompanying transition from interictal to ictal activity will be continued, using different epileptogenic agents as well as electrical stimulation.

Honors and Awards: None.

Publications: O'Connor, M., Lewis, D. and Herman C.: Effects of potassium on oxidative metabolism and seizures. Electroenceph. clin. Neurophysiol., 1973 (in press).

Serial No. NDS(I)-72 EEG/CN 1939b
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Fluorometric monitoring of intracellular redox changes in human cerebral cortex

Previous Serial Number: Same

Principal Investigator: D. V. Lewis, M.D.

Other Investigators: W. Schutte, W. Whitehouse, J. B. Van Buren, M.D., Ph. D.

Cooperating Units: Surgical Neurology Branch, IR, NINDS, Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total: 0.15
Professional: 0.10 (DVL)
Other: 0.05

Project Description:

Objectives: Evaluate the feasibility of monitoring intracellular redox changes as indicated by changes in intramitochondrial NADH and, if feasible, develop an instrument which would be both safe and convenient to use in the neurosurgical operating room. When such an instrument is functioning properly, study the energy metabolism in the human cerebral cortex and how it is affected by pathologic conditions such as epilepsy or tumors.

Methods employed: Using a recently developed television system, fluorescence changes are recorded on video tape over a wide area of brain which has been exposed in the course of neurosurgical procedures. Analysis of changes is carried out on line as well as later by video tape playback. Energy metabolism is evaluated through analysis of transients spontaneously occurring or induced by electrical stimulation.

Major findings: Reproducible changes in fluorescence induced by electrical stimulation have been obtained in a number of patients using the previous equipment. The new television system enables these responses to be analyzed topographically on the image of the exposed brain, after, and to a lesser extent, during the recording. Topographical analysis has just begun and no conclusions have been drawn.

Significance to Bio-medical Research and the Program of the Institute: It is hoped that patterns of oxidative metabolism changes will be of value in locating epileptogenic or otherwise abnormal tissue. Also these patterns might give some insight into the mechanism of epilepsy.

Proposed Course of the Project: Continued study of energy metabolism in human cortex and the ways in which it is affected by pathologic conditions.

Honors and Awards: None.

Publications: None.

Serial No. NDS(I)-72 EEG/CN 1940(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Preoperative and postoperative EEG data and electrocorticography in epileptic patients

Previous Serial Number: Same (note slight modification of title and authorship)

Principal Investigator: C. Ajmone Marsan, M.D.

Other Investigators: J. B. Van Buren, M.D., Ph.D.

Cooperating Units: Surgical Neurology Branch, IR, NINDS

Man Years:

| | |
|---|---|
| Total: | 0.45 |
| Professional: | 0.40 (CAM) |
| Other: | 0.05 |

Project Description:

Objectives: To analyze and correlate the characteristic features of preoperative EEGs and clinical state, ECoG data, and site and extent of surgical removal with postoperative EEGs and clinical results of the procedures.

Major findings: Methods, indications, possibilities, diagnostic and prognostic value of ECoG have been described and discussed. The results have been collected and are currently in press for a specialized volume of the *Handbook of Clinical Neurophysiology*. The data related to the major follow-up study of individual patients are being collected and tabulated.

Significance of Bio-Medical Research and Programs of the Institute: This project should provide useful information of diagnostic, prognostic and therapeutic significance for the surgical treatment of the epilepsies.

Proposed Course of the Project: The collection of cases and their analysis will continue at least for the whole coming fiscal period.

Honors and Wards: None.

Publications: C. Ajmone Marsan and M. J. O'Connor. Electrocortic graphy, Vol. 10 C. In Remond, A., Ajmone Marsan, C. Brazier, M. A. B., Buchthal, F., and Cobb, W. A. (eds.): Handbook of Electroencephalography and Clinical Neurophysiology. Elsevier, Amsterdam (to appear in 1973).

Serial No. NDS(I)-73 EEG/CN-2011
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Topographical analysis of cortical redox changes surrounding epileptic foci

Previous Serial Number: None

Principal Investigator: D. V. Lewis, M.D.

Other Investigators: W. Schutte, W. Whitehouse

Cooperating Units: Biomedical Engineering Branch of Division of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total: 0.50
Professional: 0.25 (DVL)
Other: 0.25

Project Description

Objectives: A topographical study of the changes in energy metabolism in a large area of cat cerebral cortex containing a focus of epileptiform activity induced by penicillin or electrical stimulation.

Methods employed: Intracellular redox changes as indicated by changes in the ratio of oxidized to reduced nicotine adenine dinucleotide (NAD) are monitored fluorometrically in vivo in the superficial cerebral cortical layers, employing a television camera and image intensifier. The resulting image of the cortex is stored on video tape along with the simultaneously recorded electrical activity. Recording is continuous during the evolution of induced epileptiform activity.

Major findings: Continuous monitoring of fluorescence changes in a large area of cerebral cortex is feasible using image intensification, appropriate filtering systems and a television camera. The image created by the fluorescence, which also displays the anatomy of the area, is stored on video tape along with simultaneous electrical activity. After experimentation, the image is displayed on a television screen by videotape playback. Changes in intensity of fluorescence in any desired area of the image can be continuously measured using a gate generator and electronic densitometer. These devices enable selection of a rectangular area of variable size and location in which light intensity is monitored. The fluorescence variation with time is displayed on graph paper with the simultaneous EEG which was recorded during the experiment. Thereby oxidative metabolism changes over the entire televised image of the cortex can be topographically analyzed and related to electrical activity. Initial results suggest that metabolic activity has different patterns at different distances from the center of a penicillin focus.

Significance of Bio-medical Research and Programs of the Institute: The mechanisms responsible for transition from interictal to ictal activity and for spread and restriction of ictal activity are not clear. This method by allowing analysis of redox changes in a focus of epileptiform activity and a large area of surrounding cortex might help elucidate these mechanisms.

Proposed Course of the Project: Continued topographical analysis of redox changes in cortex containing a epileptogenic focus. If possible, similar analysis of mirror foci.

Honors and Awards: None.

Publications: None.

Serial No. NDS(I)-73 EEG/CN 2012
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Alterations in the receptive fields of visual cortex cells associated with the development of a penicillin epileptic focus

Previous Serial Number: None

Principal Investigator: J. S. Ebersole, M.D.

Other Investigators: R. A. Levine, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.15 |
| Professional: | 1.00 |
| Other: | 0.15 |

Project Description:

Objectives: This project attempts to verify the hypothesis that the abnormal activity of a neuron in an epileptic focus is due in part to an alteration of the normal balance of excitatory and inhibitory afferents to that cell. The receptive field of a visual cortex neuron is an objectively measurable representation of that cell's inputs. The demonstration of significant changes in the characteristics of such a cell's receptive field associated with the development of an epileptic focus could clarify the relationship between altered afferent activity and epileptiform activity.

Methods employed: Cats are anesthetized with sodium pentobarbital, paralyzed with gallamine triethiodide, and artificially respired. Both eyes are refracted, and vision corrected with contact lenses. Twin glass micro-pipettes are positioned in the visual cortex (area 17). One barrel records the extracellular electrical activity of a cell, and the other is used for local micro-iontophoresis of penicillin G. Receptive fields of visual cortex neurons are characterized before, during, and after the initiation of a penicillin epileptogenic focus involving the cells under investigation.

Major findings: Preliminary results show that both firing pattern and receptive field properties are altered while a visual cortex neuron is involved in an epileptic focus. Normal activity is replaced by high frequency burst discharges (extracellular counterpart of intracellular paroxysmal depolarization shifts) that are synchronous with gross EEG "spikes." Receptive fields enlarge and lose specificity, including axis orientation and preferred movement sensitivity. A usually ineffectual flash becomes a potent stimulus. These changes, however, are not all-or-nothing, but involve intermediate stages of partial change, both during the development and decline of the epileptic focus. This intermediate stage is characterized by the presence of both normal unit spikes with intermittant burst discharges, which do not occur with every gross EEG surface discharge. Receptive fields of more normal size with only partial loss of specificity, and a lack of flash sensitivity are also present.

Significance to Bio-medical Research and Programs of the Institute: Findings to date tend to support the concept of an altered afferent bombardment upon a neuron as a factor associated with the abnormal activity of such neurons in an epileptic focus. The abnormal receptive fields of visual cortex units in a penicillin epileptic focus, including the transitional stages, have not previously been described.

Proposed Course of the Project: Refinement of present preliminary data and possible extension of the investigation to the more complex cells of the visual associative areas of the cortex (areas 18, 19).

Honors and Awards: None.

Publications: None.

Serial No. NDS(I)-73 EEG/CN 2013
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The effects of epileptogenic agents upon the single unit responses in the cerebellar cortex

Previous Serial Number: None

Principal Investigator: R. A. Levine, M.D.

Other Investigators: J. S. Ebersole, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.15 |
| Professional: | 1.00 |
| Other: | 0.15 |

Project Description:

Objectives: Epileptiform activity of the type observed in elements of the cerebral cortex and other brain structures has never been reported following the application of topical agents to the surface of the cerebellum. Likewise, the deafferented cerebellum has not shown epileptiform activity to systemic agents. The present study will examine the effect of representative epileptogenic agents upon single unit responses of the adult cat's cerebellar cortex.

Methods employed: Adult cats were anesthetized with pentobarbital. Extracellular and intracellular single unit and gross surface recordings were made from the cerebellar cortex of the anterior lobe of the vermis. A concentric electrode was placed stereotaxically in the white matter of the cerebellum for antidromic stimulation. Identification of a Purkinje cell was made on the basis of the presence of two different types of spikes in a single unit, the complex and simple spike. Antidromic stimulation and iontophoresis of a dye that could be recovered histologically were used as confirmatory evidence for the identity of Purkinje cells. Records before and after the topical application of strychnine were compared.

Major findings: Preliminary findings have shown no conclusive difference in the unit activity, either spontaneous or antidromically induced, before and after strychnine application.

Significance of Bio-Medical Research and Programs of the Institute: A knowledge of the effects of epileptogenic agents upon the neurons of the cerebellar cortex (and their characteristi synaptic circuitry) may contribute to our understanding of the mechanisms subserving epileptogenesis in general.

Proposed Course of the Project: Besides widening the data base of the present study with strychnine and thereby allowing for more critical statistical analyses of firing patterns, improved methods of applying strychnine topically, trials of other topical agents, and employment of other methods for the control of inputs to the cells of the cerebellar cortex are anticipated.

Honors and Wards: None.

Publications: None.

Serial No. NDS(I)-73 EEG/CN 2014(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical seizure patterns in patients with occipital epileptogenic foci

Previous Serial Number: None

Principal Investigator: C. Ajmone Marsan, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.35 |
| Professional: | 0.30 |
| Other: | 0.05 |

Project Description:

Objectives: To analyze the various sensory, motor autonomic and other symptoms which characterize the ictal episodes in patients with electrographic evidence of epileptiform activity in the occipital and para-occipital regions.

Methods employed: Data derived from about 50 patients with epileptic seizures selected on the basis of their EEG findings as specified above. All their records and clinical charts are reviewed and all the observed and described seizures for each subject are analyzed. The pattern(s) of each seizures and/or the various motor, sensory and other components of each ictal episode are correlated with the EEG localization and lateralization as well as with pathological, X-ray and other clinical findings.

Major findings: Project has been started recently and it is still in the phase of data collection. No major findings available as yet.

Significance to Bio-medical Research and Programs of the Institute: The understanding and treatment of epileptic disorders are among the major goals of this Institute. This project is complementary with a similar one (NDS(I)-71 EEG/CN 1868) which dealt with the seizure patterns of epileptic patients with foci on different cortical regions and was completed last year. It is expected to provide useful information on the pathophysiology of this relatively uncommon localization as well as having practical diagnostic significance and, indirectly, helping in its surgical elimination.

Proposed Course of the Project: The collection of data will continue and their analysis should be completed within one year.

Honor and Awards: None.

Publications: None.

ANNUAL REPORT
July 1, 1973 through June 30, 1973
Developmental and Metabolic Neurology Branch
National Institute of Neurological Diseases and Stroke

Roscoe O. Brady, Chief

The amalgamation of the former Section on Lipid Chemistry, LNC, with the Section on Child Neurology, SNB, occurred in FY 1973 with major benefits in several respects. A major breakthrough has been attained through the combination of basic research and clinical investigation in the new Branch. After two and one-half years of fundamental research effort, we succeeded in purifying the enzyme called ceramidetrihexosidase from human placental tissue. This enzyme is lacking in the organs and tissues of patients afflicted with Fabry's disease. The purified enzyme was pyrogen-free and was infused into two patients with Fabry's disease. The results of this therapeutic trial were very encouraging. The abnormally high level of accumulating glycolipid in the plasma of these patients was significantly lowered by administration of the enzyme. In one of the Fabry patients, the level of glycolipid fell transiently to the normal range following a single injection of enzyme. Another very promising aspect was the finding that there was enhancement of total enzymatic activity over the amount actually administered following injection into the Fabry patient. We believe that this effect is due to activation of the faulty enzyme in the patient's tissues when it becomes associated with the active injected enzyme. Taken together, these findings provide the first signs of encouragement for the success of enzyme replacement therapy for patients with certain heritable metabolic diseases.

A second major advance in a related endeavor which occurred during FY 1973 was the development of a procedure for the complete purification of the glucocerebrosidase which is lacking in patients with Gaucher's disease. After 8 years of effort, we have obtained a homogeneous preparation of this enzyme, again from human placental tissue. We are now in position to scale up the enzyme isolation procedure and then assess the efficacy of replacement therapy for patients with Gaucher's disease. This important study will again be carried out with the Clinical Investigations Section of the newly formed branch.

Another impressive advance during the past year was the documentation of the reliability of the prenatal diagnosis of Gaucher's disease and Niemann-Pick disease by enzyme assays performed on cultured amniotic cells. These contributions, coupled with our previous demonstration of the feasibility of the prenatal diagnosis of Fabry's disease, has placed our laboratory among the most frequently consulted center for genetic counseling in the world. We provide these services for any requesting physician as well as similar diagnostic tests for Tay-Sachs disease.

Work on the altered glycolipid metabolism in tumorigenic virus-transformed cells has continued satisfactorily. The specific block of ganglioside synthesis in cells transformed with oncogenic DNA viruses also occurs when cells are transformed by tumorigenic RNA viruses. Non-transforming viruses do not cause this metabolic alteration which has been shown to be due to a decrease in the activity of a specific aminosugar transferase required for the synthesis of the higher ganglioside homologs present in normal cells. Since gangliosides are highly concentrated in the plasma membrane of cells, it is likely that these components play a role in the social behavior of cells. We are assessing the contribution of these complex lipids to the phenomenon of contact inhibition of growth in culture exhibited by normal cells and the loss of this regulatory phenomenon in the tumorigenic virus-transformed cells.

The chemical synthesis of specifically labeled sphingolipids has also progressed in an exemplary fashion. The Section of Neurochemical Methodology has prepared ceramidetrihexoside, the compound which accumulates in Fabry's disease, specifically labeled with $^3$H in the terminal galactose portion of the molecule. Furthermore, the asialo-derivative of Tay-Sachs ganglioside (N-acetylgalactosaminylgalactosylglucosylceramide) has also been labeled with $^3$H in the terminal N-acetylgalactosaminyl moiety. This substance accumulates in the brain of patients with Tay-Sachs disease, but only to one-fifth the extent of the intact ganglioside (N-acetylgalactosaminyl-(N-acetylneuraminosyl)-galactosylglucosylglucosylceramide). These compounds are labeled in critical parts of the molecule for metabolic studies in the respective diseases. They are also essential substrates for monitoring the purification of enzymes which will be used in replacement therapy for these diseases.

Work on our newly-discovered myelin-specific glycoprotein has also progressed in a very satisfactory fashion. Many critical experiments which were necessary to convince ourselves of the uniqueness of this novel myelin component have all indicated that it is indeed a component of myelin. The metabolism of this protein is dramatically deranged in mutant myelin-deficient mice such as the Quaking and Jimpy strains. We now need to determine the precise role of this substance in the development of the myelin sheath and to examine its quantity and composition in demyelinating diseases of humans such as multiple sclerosis.

The efforts of the Section on Clinical Investigation in FY 1974 will be directed predominantly towards novel therapeutic trials and preventive measures in neurological diseases such as the lipidoses, mucopolysaccharidoses, and certain aspects of cerebrovascular disease and epilepsy. In addition to the direct participation in the enzyme replacement trials in Fabry's disease, during FY 1973 we demonstrated that the abnormally elevated cerebrospinal fluid content of acid mucopolysaccharides (AMPS) in Hurler, Hunter and Sanfillippo syndromes correlates positively with the profound mental deterioration and neurological symptoms in these patients whereas normal CSF content of AMPS in another mucopolysaccharidosis - Scheie disease - is associated with normal brain function in these patients Moreover, it appears that the specific AMPS-heparitin sulfate-is the substance responsible for the brain abnormality; this lead will be further investigated.

Therapeutic trials in several other genetic disorders with neurological involvement were conducted. Repeated infusions of fresh human plasma and whole blood to patients with various mucopolysaccharidoses had little effect on their clinical course of the excretion of AMPS. However, alternate-day prolonged administration of corticosteroids was associated with definite clinical amelioration.

A complete chemical analysis of the composition of AMPS in the renal cortex and medulla of normal human kidneys and kidneys from Hurler and Hunter patients has been carried out for the first time. The kidneys from these patients had a 40-60-fold increase in AMPS content over the normal control level; also, the AMPS in the patients' kidneys was greatly degraded. These findings will be useful in further studies of these disorders, especially with regard to therapeutic trials to increase the excretion of the accumulated substances.

A patient with catamenial epilepsy was successfully managed by administration of progesterone in the form of a long-lasting depot injection. Further studies in this promising direction will be conducted. Other studies are in progress on the level and mode of action of anticonvulsant drugs and they will be extended during the forthcoming year.

Qualitative and quantitative determinations of acidic proteins were investigated in fresh human brain cortex and white matter. The samples were obtained by biopsy or during surgical procedures in disorders such as progressive cerebral degeneration and gliomas of the brain. In some gliomas, and in certain other conditions associated with gliosis of the brain, a heavy protein band appeared in polyacrylamide gels which was not detected in specimens of normal brain tissue. The characteristics and properties of this protein will be examined in a detailed investigation of this component.

In summary, the combination of basic science capability with patient investigation facilities has provided an impressive opportunity for accelerating research in the treatment of hereditary diseases. The contributions of the branch which were reported in 45 publications emanating from this unit in FY 1973 document the benefit and wisdom of this combined research endeavor.

Serial No. NDS(I)-60 DMNB/ND 706(c)
1. Developmental and Metabolic Neurology Branch
2. Section on Neurological Development
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical, Genetic, Biochemical and Pathological Studies of Progressive Cerebral Degenerations, Mental Retardation and Birth Defects; with Emphasis on Familial Occurrence.

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: George Constantopoulos, Ph.D. and Andrew W. Zimmerman, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 2.5 |
| Professional: | 1.8 |
| Other: | 0.7 |

Project Description:

Objectives: The majority of chronic neurological disorders affect patients from their early years of life and constitute one of the greatest medical and social problems of our times. According to conservative estimates for this country, over four million people are permanently handicapped by mental retardation (frequently of familial type), birth defects, epilepsy and metabolic (genetic) disorders affecting the nervous system. Our main objectives are: a) to identify those conditions with neurological involvement which have increased familial incidence; b) to separate these patients into probable hereditary and acquired categories; c) to study the pathogenesis and etiology of certain of these conditions; d) to devise special diagnostic tests and e) to institute therapeutic modifications in several groups of these diseases and to explore preventive measures.

Methods Employed:

A. Diagnostic

1. Detailed general medical, neurological, psychological and developmental studies.

2. Genetic (pedigree type), cytogenetic and biochemical-genetic studies.
3. Special biochemical assays of blood, CSF, urine and saliva which permit general assessment of protein, carbohydrate, lipid, glycolipid and nucleoprotein metabolism as well as the state of immunological responses.
4. Employment of special procedures when indicated, such as pneumoencephalography and arteriography, and in special instances, brain biopsy.
5. Employment of special enzymatic assays using patients' white blood cells, plasma, fibroblast tissue cultures and, occasionally, biopsy of liver, gut or brain.

B. Therapeutic

1. Use of approved and investigative pharmacological agents employing modifications of the drugs, dosages and others.
2. Use of corticosteroids for suppressing inflammatory reaction or enhancement of enzymatic activity.
3. Use of plasma, whole blood or white blood cells (obtained by plasmapheresis), for intravenous infusions as means of supplying deficient enzymes.
4. Identification, isolation, purification and intravenous infusion of the respective deficient enzymes in special categories of genetic disorders.

Patient Material: 26 inpatients, many of whom were admitted several times (a total of 860 patient days) and 19 outpatients were studied under this project.

Major Findings:

I. In order to supply presumed deficient enzymes to patients with Hurler Hunter and Sanfilippo variants, we have carried out repeated transfusions of fresh plasma and whole blood in large quantities. Results: clinical observations and total 24-hour urinary AMPS and their composition and molecular weight distribution were determined before, during and after transfusions. The two patients who received plasma transfusions showed no noticeable change in the amount of AMPS excreted; of the three patients who received whole blood transfusions, two had slightly less excretion of AMPS while the third showed no difference. The AMPS in the CSF were measured in one patient before and after blood transfusions and found to be unchanged; likewise, the determination of molecular weights in the isolated AMPS was virtually identical. However, alternate day prolonged administration of corticosteroids to several patients with Hurler, Hunter, and Sanfilippo variants decreased by 30-40 per cent urinary excretion of mucopolysaccharides and afforded noticeable improvement in their clinical disability.

II. More favorable were our therapeutic trials in the patients with Fabry's disease. This is a genetically determined metabolic disorder characterized clinically by progressive renal damage, corneal opacity, peripheral neuropathy and emotional changes; the biochemical abnormality consists of excessive accumulation of ceramide trihexoside in various organs and deficiency of enzyme α-galactosidase activity. In order to supply the missing enzyme, fresh human plasma (680 ml) was infused to the patients. This caused only a small decrease in the amount of circulating globoside. However, subsequent infusion of fresh WBC-platelet enriched plasma obtained by plasmapheresis had definite effect: the level of α-galactosidase activity in the patient's serum rose to 68% over the pre-infusion level and persisted for four hours, declining gradually during the subsequent 20 hours. Of greater importance was the finding that plasma ceramide trihexoside concentration (accumulating substrate) had decreased by nearly 50 per cent. It is of interest that I.V. infusion of 5 mg of the highly purified enzyme (α-galactosidase) gave essentially similar results as infusion of WBC + platelets. These results are the first ever obtained and mark a new era in therapeutic approaches to certain hereditary disorders.

III. A multidisciplinary approach was used in the study of families with Laurence-Moon syndrome, a hereditary conditions characterized by mental deficiency, polydactyly, pigmentary degeneration of the retina, obesity and gonadal dysfunction. The major findings are as follows: a) the defective brain function is likely to be related to hypothalamo-endocrinological dysfunction; b) the delayed puberty is common, however, true hypogonadism is low and has been erroneously stressed in medical literature as a common feature; c) on the basis of pedigree evaluation of 13 families we postulate existance of different genetic types of the syndrome, since intrafamily concordance of major abnormalities was the usual finding while there was a marked interfamilial variability; d) first demonstration of renal caliceal abnormality of developmental origin.

IV. Monozygotic twins provide a very useful source for evaluation of genetic and environmental influences on normal and abnormal development. We have studied a set of twins who were concordant for duplicated urinary collecting systems and for mental subnormality. Subsequently, one of these twins had head injury followed by hemiparesis and epilepsy which provided interesting encephalographic data. This was the first set of twins reported who were concordant for duplicated collecting systems and also the first to have this associated with mental subnormality. We are in need of additional concordant twins with organic congenital abnormalities and associated brain dysfunction (mental retardation in particular) for exploration of common pathogenic hypothesis acting during embryogenesis.

Significance to Bio-Medical Research and the Program of the Institute: There are over one hundred crippling hereditary disorders and they affect usually multiple organs; however, either directly or indirectly the nervous system is involved in nearly all of them. The results presented here advance our knowledge in the genetic mechanisms of some of these disorders. Therapeutic modifications of the course of the disease and changes in metabolism are likely to provide us with better understanding of these disorders and their eventual management.

Proposed Course of the Project: Our efforts will continue in determinations of pathogenesis and etiology of individual genetic disorders. The considerable success in preliminary therapeutic trials in mucopolysaccharidosis and Fabry's disease would be extended using improved approaches as we learn more; similar attempts will be extened to other hereditary diseases.

Honors and Awards: Clinical Associate Professor of Neurology, George Washington University Medical School (no remuneration).

Publications:

Dekaban, A.S., Holden, K.R. and Constantopoulos, G.: Effects of fresh plasma or whole blood transfusions on patients with various types of mucopolysaccharidosis. Pediatrics 50: 688-692, 1972.

Dekaban, A.S., Parks, J.S. and Ross, G.T.: Laurence-Moon syndrome: evaluation of endocrinological function and phenotypic concordance and report of cases. Med. Ann. D.C. 41: 687-693, 1972.

Holden, K.R. and Dekaban, A.S.: Concordance of duplicated urinary collecting systems and mental subnormality in monozygotic twins. Clin. Proc. Child. Hosp. D.C. (in press).

Serial No. NDS(1)-61 DMN/EG 815
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Metabolism of Complex Lipids of Nervous Tissue, Studies on Gaucher's Disease, Niemann-Pick Disease, Fabry's Disease, Krabbe's Disease and Metachromatic Leukodystrophy.

Previous Serial Number: Same.

Principal Investigator: Peter G. Pentchev, Ph.D.

Other Investigators: Roscoe O. Brady, M. D., A. E. Gal, Ph.D., John F. Tallman, Ph.D.; George E. Mook and Sue R. Hibbert.

Cooperating Units: Weizmann Institute of Science, Rehovot, Israel.

Man Years:

| | |
|---|---|
| Total: | 4.8 |
| Professional: | 2.3 |
| Other: | 2.5 |

Project Description:

Objectives: (1) To elucidate the biosynthetic pathways for the formation of long chain fatty acids, cerebrosides, gangliosides, and sphingomyelin; (2) to study the control mechanisms which regulate these processes; (3) to study the metabolic fate of sphingolipids in normal and lipodystrophic disease states, and (4) to provide diagnostic and therapeutic procedures for the amelioration and control of the lipid storage diseases.

Methods: Glucocerebroside and galactocerebroside labeled with $^{14}C$ in either the hexose or fatty acid portion of the molecule have been synthesized. $^{14}C$-labeled sphingomyelin and gluco-and galactopsychosine have been similarly prepared. Ceramide-trihexoside and ceramide tetrahexoside (globoside) uniformly labeled with radioactive hydrogen-$^{3}H$ have been prepared. The metabolism of these labeled materials has been investigated in vivo and in vitro.

Major Findings: During the past year we accomplished three major breakthroughs in our investigations in the sphingolipidoses. 1. After eight years of work, we have succeeded in completely purifying glucocerebroside, the enzyme involved in Gaucher's disease. Homogeneous preparations of this enzyme have now been obtained from human placental tissue. 2. We have also purified ceramidetrihexosidase, the enzyme involved in Fabry's disease, from the same tissue source. 3. The latter enzyme has been successfully infused into two patients with Fabry's disease. The elevated level of ceramidetrihexoside in the blood stream of the patients with Fabry's disease was lowered into the range in normal humans by this enzyme replacement procedure.

We continue to serve as a center for the diagnosis of patients and detection of carriers for all of the lipid storage diseases. Requests and samples come from all over the world for these assays. Much current work is devoted to the monitoring of pregnancies at risk for these heritable metabolic disorders. During the past year, we conclusively identified four fetuses with Niemann-Pick disease, three with Gaucher's disease and one with Fabry's disease. Other fetuses were normal or symptomatically unaffected heterozygotes like the parents.

Significance: The results obtained from enzyme infusion in patients with Fabry's disease are very encouraging. It seems very Likely that enzyme replacement therapy will be of considerable benefit to patients with this disease.

Proposed course: We will undertake an investigation of the long term effect of enzyme administration on the clinical course of Fabry's disease. We will also investigate the effect of enzyme replacement therapy in patients with Gaucher's disease now that we have obtained a completely purified preparation of the requisite glucocerebrosidase.

Publications:

1. Johnson, W. G. and Brady, R. O.: Ceramide trihexosidase from human placenta. In Ginsburg, V. (Ed.): Methods in Enzymology, Vol. XXVIII, Pt. B. New York, Academic Press, 1972, pp. 849-856.

2. Weinreb, N. L., and Brady, R. O.: Glucocerebrosidase from bovine spleen (cerebroside-β-glucosidase). In Ginsburg, V. (Ed.): Methods in Enzymology, Vol. XXVIII, New York, Academic Press, 1972, pp. 830-834.

3. Brady, R. O., and King, F.: Gaucher's disease. In Hers, H. G. and Van Hoof, F. (Eds.): Liposomes and Storage Diseases. New York, Academic Press, 1973, pp. 381-394.

4. Brady, R. O., and King, F.: Niemann-Pick disease. In Hers, H. G. and Van Hoof, F. (Eds.): Lysosomes and Storage Diseases. New York, Academic Press, 1973, pp. 439-452.

5. Schneider, E. L., Ellis, W. G., Brady, R. O., McCulloch, J. R., and Epstein, C. J.: Prenatal Niemann-Pick disease; biochemical and histological examination of a 9-gestational week fetus. Pediatr. Res. 6: 720-729, 1972.

6. Schneider, E. L., Ellis, W. G., Brady, R. O., McCulloch, J. R., and Epstein, C. J.: Infantile (Type II) Gaucher's disease: In utero diagnosis and fetal pathology. J. Pediatr. 81: 1134-1139, 1972.

7. Brady, R. O.: Biochemical basis of familial sphingolipidoses. Semin. Hematol. 9: 273-284, 1972.

8. Brady, R. O.: The sphingolipodystrophies. In Bondy, P. K., and Rosenberg, L. E. (Eds.): Duncan's Diseases of Metabolism, Seventh Edition, Philadelphia, W. B. Saunders Co., 1973, in press.

9. Brady, R. O.: Hereditary diseases, causes, cures and problems. Angew. Chem. 85: 28-38, 1973. Internat'l. English Ed., 12: 1-11, 1973.

10. Brady, R. O.: Lipidoses. Biochimie. 54: 723-733, 1972.

11. Pentchev, P. G., and Brady, R. O. The effect of a heat-stable factor in human spleen on glucocerebrosidase and acid beta-glucosidase activities. Biochim. Biophys. Acta 297: 491-496, 1973.

12. Brady, R. O.: Physiologic considerations of tissue lipids. Ann. Clin. Lab. Sci. 2: 132-136, 1972.

13. Brady, R. O.: Enzyme defects in the sphingolipidoses and their application to diagnosis. Ann. Clin. Lab. Sci. 2: 285-294, 1972.

14. Desnick, S. J., Desnick, R. J., Brady, R. O., Pentchev, P. G., Simmons, R. L., Najarian, J. S., Swaiman, K., Sharp, H. L., and Krivit, W.: Renal transplantation in type 2 Gaucher's disease. In Desnick, R. J., Bernlohr, R. W., and Krivit, W., (Eds.): Birth Defects Original Article Series, Vol. 9, 1973, in press.

15. Brady, R. O.: Sphingolipidoses. In Albers, R. W., Siegel, G. J., Katzman, R., and Agranoff, B. W. (Eds.): Basic Neurochemistry. Boston, Little, Brown and Co., 1972, pp. 485-495.

16. Brady, R. O.: Disorders of lipid metabolism. In Smellie, R. M. S., (Ed.): Current Trends in the Biochemistry of Lipids. London, Academic Press, Inc., 1973, pp. 113-127.

Serial No. NDS(1)-61 DMN/EG 816
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Structural and Metabolic Studies of Gangliosides in Normal Humans and Patients with Tay-Sachs Disease.

Previous Serial Number: Same.

Principal Investigator: John F. Tallman, Ph.D.

Other Investigators: R. O. Brady, M.D., Andrew E. Gal, Ph.D.; Jane M. Quirk.

Cooperating Units: None.

Man Years:

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|---|---|
| Total: | 2.3 |
| Professional: | 1.3 |
| Other: | 1.0 |

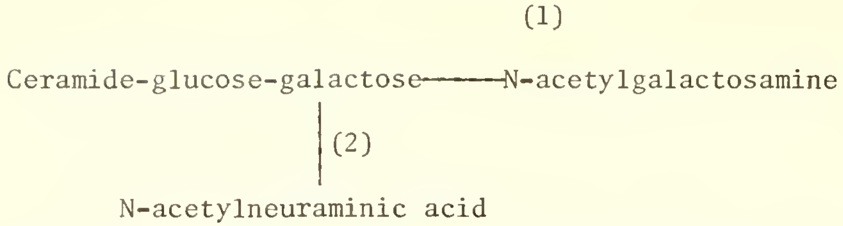
Project Description:

Objectives: To investigate the involvement of the hexosaminidases in the metabolism of gangliosides and their derangement in patients with Tay-Sachs disease.

Methods: Tay-Sachs ganglioside N-acetylgalactosaminyl-(N-acetylneuraminosyl)-galactosyl-glucosylceramide was labeled in the N-acetylgalactosaminyl portion of the molecule with $^{14}C$ through an enzymatic synthetic scheme utilizing UDP-N-acetylgalactosamine-1-$^{14}C$ and a soluble galactosaminyl transferase from rat brain. The asialo derivative ($GA_2$) of this compound was labeled with tritium by galactose oxidase treatment followed by reduction with tritiated sodium borohydride. The N-acetylneuraminic acid labeled Tay-Sachs ganglioside was prepared biosynthetically from precursor N-acetylmannosamine-$^{3}H$.

Major Findings: The site of the metabolic defect in Tay-Sachs disease has now been conclusively identified in brain with the use of Tay-Sachs ganglioside specifically labeled in the N-acetylgalactosaminyl portion of the molecule. There is complete absence of the enzyme hexosaminidase which catalyzes the cleavage of the terminal molecule of N-acetylgalactosamine from Tay-Sachs ganglioside in the tissues of patients with this disease (Site 1). Sialidase activity (Site 2) is normal in patients with Tay-Sachs disease.

# TAY-SACHS GANGLIOSIDE



We have purified hexosaminidases A and B from human placenta and are examining the kinetics and physical structure of these purified enzymes. They are closely related to each other and the deficit of one of these enzymes (A) yields the pathological condition known as Tay-Sachs disease. A deficiency of both enzymes forms has been noted on the O-variant of Tay-Sachs disease.

We have also purified hexosaminidase A from human urine. A portion of the purified urinary enzyme was administered intravenously to a patient with the O-variant form of Tay-Sachs disease (total hexosaminidase-deficiency). The kinetics of the clearance of the enzyme from the blood stream were determined and the amount of enzyme which entered the brain and liver were determined by biopsying the respective organs.

Significance: The demonstration of the nature of the metabolic defect in Tay-Sachs disease now permits the consideration of rational therapeutic measures for attempts at alleviating this disorder. Furthermore, tests have been developed for the early diagnosis of infants as well as the antenatal detection of fetuses affected with Tay-Sachs disease. In the past year we have carried out over 125 tests for patients and carriers of Tay-Sachs disease.

Proposed Course: We shall extend our work on the purified enzyme and therapeutic trials. Examination of the physical properties of these two enzymes and their respective substrate specifications should lead us to a complete understanding of the molecular pathology of Tay-Sachs disease.

Publications:

1. Brady, R. O., Tallman, J. F., Johnson, W. G., and Quirk, J. M.: An investigation of the metabolism of Tay-Sachs ganglioside specifically labeled in critical portions of the molecule. In: Sphingolipids, Sphingolipidoses, and Allied Disorders, Advances in Exptl. Med. and Biol. 19: 277-286, 1972.

2. Quirk, J. M., Tallman, J. R., and Brady, R. O.: Preparation of trihexosyl and tetrahexosyl gangliosides specifically labeled in the N-acetylgalactosaminyl moiety. J. Labelled Compounds 8: 483-494, 1972.

3. Tallman, J. F. and Brady, R. O.: Neuraminidase (sialidase) from rat heart. In Ginsburg, V. (Ed.): Methods in Enzymology, Vol. XXVIII, Pt. B, New York, Academic Press, 1972, pp. 825-829.

4. Tallman, J. F., Johnson, W. G., and Brady, R. O.: The metabolism of Tay-Sachs ganglioside: Studies with lysosomal enzymes from normal and Tay-Sachs brain tissue. J. Clin. Invest., 51: 2339-2345, 1973.

5. Tallman, J. F., and Brady, R. O.: The catabolism of Tay-Sachs ganglioside by rat brain lysosomes. J. Biol. Chem. 247, 7570-7575, 1972.

6. Tallman, J. F., and Brady, R. O.: Purification and properties of a mammalian neuraminidase (sialidase). Biochim. Biophys. Acta., 273: 434-443, 1973.

7. Tallman, J. F., Kolodny, E. H., and Brady, R. O.: Tay-Sachs ganglioside specifically labeled in either the N-acetylneuraminosyl or galactosaminyl moiety. In Lowenstein, J. M. (Ed.): Methods in Enzymology, Vol. XXVIII, Pt. II, in press, 1973.

8. Johnson, W. G., Mook, G., and Brady, R. O.: β-Hexosaminidase A from human placenta. In Ginsburg, V. (Ed.): Methods in Enzymology, Vol. XXVIII, Pt. B, New York, Academic Press, 1972, pp. 857-861.

9. Johnson, W. G., Desnick, R. I., Long, D. M., Sharp, H. L., Krivit, W., Brady, B. and Brady, R. O.: Intravenous injection of purified hexosaminidase A into a patient with Tay-Sachs disease. National Foundation Original Article Series, 1973, in press.

Serial No. NDS(I)-63 DMNB/ND 1026(c)
1. Developmental and Metabolic Neurology Branch
2. Section on Neurological Development
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical and Biochemical Studies on Epilepsy

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Ken Fujitani, M.D., Andrew W. Zimmerman, M.D. and George Constantopoulos, Ph.D.

Cooperating Units: None

Man Years:

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|---|---|
| Total: | 1.5 |
| Professional: | 1.3 |
| Other: | 0.2 |

Project Description:

Objectives: There are close to 2 million people in the U.S. who are subject to epileptic attacks due to various causes. The onset of seizures is during childhood in over 65 per cent of these patients. Idiopathic (cryptogenic) epilepsy is the most common. Thus, there is a great need for broad as well as specific studies in the field of epilepsy, especially at the time of its onset. In the first place we wish to obtain a better understanding of the underlying mechanisms of epileptic seizures and improved therapeutic procedures; this eventually should be followed by suitable preventive measures. Our present specific approaches consist of employment of high fat diet for control of seizures during early childhood and of progesterone for catamenial exacerbation of seizures. Elucidation of the mechanism of action of these regimens will be carried out in both patients and in laboratory animals. In addition, we have originated studies on altered modes of metabolism of anticonvulsant drugs in different patients, especially depending on age and genetic constitution.

Patient Material: 18 inpatients (345 patient days) and 18 outpatients.

Methods Employed:
1. General medical, developmental and neurological examinations including routine and special laboratory tests.
2. Modified electroencephalographic studies and special performance testing.

3. Pneumoencephalography and arteriography, when indicated.
4. Determination of partitioned amino acids and lipids as well as assays of carbohydrate and endocrinological function.
5. Application of special therapeutic procedures including ketogenic diet and use of certain hormones (progesterone, corticosteroids).
6. Determination of anticonvulsant drugs in serum, CSF and urine during modified metabolic states and while on different doses.

Major Findings:

1. The incidence and pathogenesis of epilepsy was investigated in a syndrome known as "Congenital retinal blindness"; this is a recessively inherited disorder which accounts for about 10 per cent of blind children. We have contributed significantly to the clarification of the pathology of this condition during the past few years. Of special interest here is the finding that a large proportion of these patients have severe neurological abnormalities and a sizeable proportion suffer from recurrent epileptic attacks. Out of 13 patients whom we studied personally, 45% had epilepsy. We have demonstrated microscopically that the retina of these patients shows complete lack of cones and rods and has deficient cell population in the external granular layer. This we interpret as congenital dysgenesis of the retina. The examination of the cerebral cortex from two of these patients showed imperfect maturation of the neurons with increased ratio of the nucleus to the cytoplasm. The relation of these neuronal changes to recurrent cerebral seizures will be further investigated.

2. The association of an increase in seizure frequency prior to or during menses occurs in about 50% of epileptic women; many neurologists refer to this as "catamenial" epilepsy. The mechanism and physiopathology of the exacerbation of seizure activity are unknown; water retention and electrolyte changes were suggested by findings of certain investigators but disproved by others. We have studied several such patients but particularly intensively an eight year old Caucasian female with premature puberty and severe exacerbation of epileptic attacks prior to and throughout her menstrual period, which generally lasts about 7 days. During this time, she has also unmanageable bad behavior and abusive language. When various combinations of anticonvulsant drugs failed to control the frequent seizures, we decided to stop her menstrual cycles. She was given three bi-weekly intramuscular injections of depot-preparation of progesterone (Provera 250, 250 and 150 mg). This brought cessation of menses for six months and initial reduction of seizures followed by complete freedom of attacks for 4 months. Her behavior and school performance has greatly improved. Seven months after the Provera injections she began to have "spotting" along with 1-3 seizures daily. We plan to explore further the mechanism of action of progesterone and estrogen in other patients.

3. There are three principal modes of action of anticonvulsant drugs; a- stabilization of the neuronal membranes; b- decrease of repetitive discharge; c- reduction of the spread of seizure discharges. Certain of

the anticonvulsants possess one or another mode of action predominantly. Dilantin, for example, is most effective in reducing spread of the discharge while Phenobarbital influences all three mechanisms but also depresses the CNS generally. Using various doses of the same two or three anticonvulsants drugs given in combination to 14 patients, we have studied the individual variation in metabolism of these drugs by determining their levels in the serum and CSF as well as their content in 24-hour urine. Preliminary results show excessive nonlinear increase in serum and CSF levels of Phenobarbital when this drug is increased by a defined ratio of the basal (conventional) dose. Also it is known that a substantial portion of Primidone is promptly converted by the organism to Phenobarbital. Factorial increase of the Primidone dose is followed by the lower ratio of its conversion to Phenobarbital. These and other data in progress will help to bring about a long overdue change in the hitherto subjective approach to objective laboratory and clinical assessment - a state which would greatly benefit the majority of epileptic patients.

Significance to Bio-Medical Research and the Program of the Institute: Our understanding of the pathogenesis of epileptic seizures and their control is still meager. Many young children become severely and permanently retarded as a result of frequent seizures. Biochemical approach to epilepsy is likely to provide us with better understanding of this syndrome complex and it may help us in the therapy of this distressing disorder. Present investigations enlarge the overall scope of the study on epilepsy which is one of the major research efforts of NINDS.

Proposed Course of the Project: During the coming year the physiological and biochemical aspects of the mechanism of action of high fat diet in children with epilepsy will be expanded. The metabolic studies on the anticonvulsant drugs are satisfactorily progressing. Experimental approach to optimal dosage will be developed during the coming year.

Honors and Awards: None

Publications:

Dekaban, A.S.: Mental retardation and neurological involvement in patients with congenital retinal blindness. Develop. Med. Child. Neurol. 14: 436-444, 1972.

Serial No. NDS(1)-66 DMN/EG 1309
1. Developmental and Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on the Metabolism of Sphingolipids in Tumor Tissues.

Previous Serial Number: Same.

Principal Investigator: P. H. Fishman, Ph.D.

Other Investigators: R. O. Brady, M.D., P. L. Coleman, Ph.D.; R. M. Bradley.

Cooperating Units: National Cancer Institute.

Man Years:

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| Total: | 2.6 |
| Professional: | 2.3 |
| Other: | 0.3 |

Project Description:

Objectives: To determine the mechanism responsible for the altered glycosphingolipid metabolism in neoplastic tissues.

Methods: The metabolism of glycosphingolipids is studied in normal contact-inhibited and tumorigenic virus-transformed cells in tissue culture. Catabolism is measured *in vitro* with specifically labeled ($^{14}$C or $^{3}$H) glycosphingolipids prepared in our laboratory. Biosynthesis is determined by labeling the sphingolipids with $^{14}$C-or $^{3}$H-labeled precursors added to the culture medium. *In vitro* biosynthesis is detected by the activities of the various glycosyltransferases involved in glycosphingolipid formation. Similar studies are carried out on spontaneously and chemically transformed cells in culture.

Major Findings: Cell lines transformed by oncogenic DNA viruses have an altered ganglioside composition which is characterized by a diminution of the N-acetylgalactosamine containing gangliosides. This change is caused by a block in the enzyme which catalyzes the synthesis of these complex lipids, hematoside: UDP-N-acetylgalactosaminyltransferase. The block in this enzyme activity appears to be specific as other glycosyltransferases involved in ganglioside biosynthesis are not blocked. The change in ganglioside metabolism is a stably inherited property of transformed cells.

Normal cells infected with the transforming RNA virus, Moloney sarcoma virus, are completely transformed within one to two days. There is a reduction in N-acetylgalactosaminyltransferase activity in these cells following transformation whereas other ganglioside glycosyltransferases are not reduced. Ganglioside analysis of these newly transformed cells shows an altered ganglioside composition similar to that found in DNA virus-transformed cells. The non-transforming Moloney leukemia virus had no effect on ganglioside metabolism in these cells. This enzymatic change, reduction in the key aminosugar transferase, is the first clearly delineated biochemical change occurring in viral transformation which appears to be common to both RNA and DNA tumor viruses.

Significance: Elucidation of the control processes which bring about depression and restoration of this ganglioside synthesizing enzyme is potentially very important for obtaining insight into metabolic changes that occur in oncogenesis.

Proposed Course: Extension of these studies to spontaneously and chemically transformed cells. Exploration of the regulatory mechanisms involved in glycosphingolipid metabolism. Elucidation of the mechanism by which tumor viruses can alter glycosphingolipid metabolism in neoplastic cells through depression of amino sugar transferase activity.

Publications:

1. Fishman, P. H., McFarland, V. W., Mora, P. T., and Brady, R. O.: Ganglioside Biosynthesis in Mouse cells: Glycosyltransferase Activities in Normal and Virally-transformed lines. Biochem. Biophys. Res. Commun. 48: 48-57, 1972.

2. Brady, R. O., Fishman, P. H., and Mora, P. T.: Membrane Components and Enzymes in Virally Transformed Cells. Fed. Proc. 32: 102-108, 1973.

3. Brady, R. O., Fishman, P. H., and Mora, P. T.: Alterations of Complex Lipid Metabolism in Tumorigenic Virus Transformed Cell Lines. Adv. Enzyme Regul. 11: 231-251, 1973.

4. Fishman, P. H., Brady, R. O., and Mora, P. T. : Altered Glycolipid Metabolism Related to Viral Transformation of Established Mouse Cell Lines. In Wood, R. (Ed.): Tumor Lipids: Biochemistry and Metabolism. American Oil Chemist's Society, 1973, in press.

5. Brady, R. O., and Fishman, P. H.: Alterations in Membrane Glycolipids in Tumorigenic Virus-Transformed Cell Lines. In Kent, P. W. (Ed.): Membrane Mediated Information: Function and Biosynthesis of Membrane Lipids and Glycoproteins. London, Oxford University Press, 1973, in press.

6. Brady, R. O., and Fishman, P. H.: Membranes of Transformed Mammalian Cells. In Fox, C. F. (Ed.): Biochemistry of Cell Walls and Membranes, MTP Biochemistry Series, Oxford, Medical and Technical Publishing Co., Oxford, 1973, in press.

7. Brady, R. O.: Alterations of complex lipid metabolism in tumorigenic virus-transformed cells. In Day, S. B., and Good, R. A. (Eds.): Membranes and Viruses in Immunopathology. New York, Academic Press, 1972, pp. 367-379.

Serial No. NDS(1)-67 DMN/NM 1457
1. Developmental & Metabolic
Neurology Branch
2. Neurochemical Methodology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Chemical Synthesis of Radioactive Sphingolipids.

Previous Serial Number: Same.

Principal Investigator: A. E. Gal, Ph.D.

Other Investigators: Frank J. Fash.

Cooperating Units: None.

Man Years:

| | |
|---|---|
| Total: | 1.5 |
| Professional: | 0.5 |
| Other: | 1.0 |

Project Description:

Objectives: To prepare sphingolipids labeled with radioactive isotopes. These compounds are used for metabolic studies or as diagnostic tools in investigations related to hereditary lipid storage diseases.

Methods and Major Findings: A multitude of approaches were used to effect the labeling of sphingolipids. Most of our effort was directed however toward specific labeling. Using this approach only a predetermined part or atom of a complex molecule will be labeled. To accomplish this the synthesis of an entire molecule is the best approach but unfortunately the synthesis of only the simplest glycolipids such as ceramide mono or dihexoside is known today. Following this approach the synthesis of sphingosine and galactocerebroside was repeatedly done by us. Partial synthesis is another approach whereby only a part of the molecule is synthesized. An example of this would be a procedure whereby a part of the lipid isolated from autopsy material is oxidized and then reduced with a tritiated reducing agent. Numerous sphingolipids such a ceramide trihexoside, asialo Tay Sach ganglioside, Tay Sach ganglioside were prepared this way in radioactive forms. A new approach based on the exchange of an entire functional group (such as an acetyl) was originated here and is studied by us. This would allow the labeling of complex gangliosides which can not be labeled specifically by chemical techniques. Non-specific labeling, the fourth approach was accomplished repeatedly in cases where specific labeling could not be done. An example of this is the labeling with tritium gas with or without a catalyst known as the Wiltzbach technique.

Significance: Prenatal diagnosis and the identification of heterozygous carriers are getting more and more important in the study and treatment of lipid storage diseases. Radioactive substrates are an indispensable tool in this endeavor. The availability of these compounds is restricted to research centers such as ours where intense work is performed in this field. It would be of great interest to develop new methods which would allow to prepare relatively easily and inexpensively these highly complex products and making them more available to researchers and clinicians.

Proposed course: By using the above discussed techniques different glycolipids used in the studies connected to lipid storage diseases will be labeled with tritium and $^{14}C$. The technique based on enzymatic oxidation and reduction with tritiated reagent will be extended to other until now, not labeled lipids. Also possible ways will be explored with the aim of replacing the enzymatic with chemical oxidation. The labeling by functional group exchange (which has a great future potential) will be studied more intensely.

## Publications

None.

Serial No. NDS(I)-68 DMNB/ND 1523(c)
1. Developmental and Metabolic Neurology Branch
2. Section on Neurological Development
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neurological, General Medical and Biochemical Aspects of Hurler, Hunter, Sanfilippo and Scheie Diseases: Basic Pathogenesis, Enzymology and Classification Criteria.

Previous Serial Number: Same

Principal Investigators: Anatole S. Dekaban, M.D., Ph.D. and George Constantopoulos, Ph.D.

Other Investigators: Dennis F. Cain, Ph.D. and Marvin Zelkowitz, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.9 |
| Professional: | 1.5 |
| Other: | 0.4 |

Project Description:

Objectives: The disorders of the metabolism of acid mucopolysaccharides are usually associated with severe dysfunction of the nervous system as well as liver, spleen, heart and others. Within the past 8 years, several genetically different diseases were identified and our Unit provided sizeable contributions in delineating certain of these entities and pioneered in attempts at modifying the course of these diseases. The interest in these disorders is additionally increased by the fact that the derangement of the CNS relates to the excessive accumulation of lipid-staining substance in the neurons (leading eventually to their destruction), while liver, spleen, skin, kidneys and other organs store mucopolysaccharides. The pathogenesis and development of new diagnostic and enzymatic approaches will be investigated with a view to establish rational therapeutic trials.

Patient Material: 14 inpatients (254 patient days) and 12 outpatients.

Methods Employed:

1. General medical, developmental and neurological exminations including special tests as required.
2. Isolation, separation and determination of composition of various

mucopolysaccharides in urine, serum and cerebrospinal fluid.

3. Determination of the molecular weight distribution in the isolated mucopolysaccharides.
4. Establishment of skin fibroblast tissue cultures for the purpose of histochemical studies and chemical determination of AMPS.
5. Utilization of tissues from organs obtained by biopsy for determination of acid mucopolysaccharide composition and enzyme studies.
6. Extraction of tissue from organs obtained at autopsy for large scale recovery of the stored acid mucopolysaccharides and lipids.
7. Production of biologically labelled $^{35}S$ heparitin sulfate utilizing skin fibroblasts in the tissue culture from patients with Sanfilippo A variant. This labelled substrate will be then used for determination of respective enzymatic activity in leukocytes and plasma of patients and unaffected controls.

Major Findings:

1. The content and composition of acid mucopolysaccharides in the cerebrospinal fluid was studied in 14 patients and in pooled normal controls. Three patients belong to type I (Hurler), two to type II (Hunter), six to type III (Sanfilippo) and three to type V (Scheie). The principal new findings consist of the markedly elevated mucopolysaccharides in the cerebrospinal fluid of the patients with type I through III, while the patients of type V had normal levels of mucopolysaccharides in their CSF. Since the patients of type V were also the only ones who had normal intellect, a positive correlation between the high content of mucopolysaccharides in the CSF and progressive mental deterioration is postulated. Moreover, it was the heparitin sulfate which accounted for the greatly elevated mucopolysaccharides in the CSF. In this context it should be stressed that patients with type III (Sanfilippo), whose major disability is mental deterioration, excrete in their urine heparitin sulfate in the range of 80-95% of all mucopolysaccharides. Further studies in this area may prove very fruitful and they are in progress.

2. Since the patients with mucopolysaccharidoses excrete excessive amount of AMPS in their urine, it was important to determine the content and distribution of these compounds in kidneys. Hurler's kidneys had 50-60-fold increase in the chondroitin sulfate B content and 10-fold increase in the Hunter kidney. The ratios of heparitin sulfate to chondroitin sulfate B in the kidneys of these patients parallel almost exactly the respective ratios in their urine. Another finding of significance relates to the large shift of the molecular weight towards smaller molecules, thus indicating that some or all of the AMPS from patients' kidneys have been considerably degraded and consists of fragments of the macromolecules.

3. Utilizing brain biopsy from the Sanfilippo patient and brain tissue from autopsy of the Hurler patient, determination of lipids and gangliosides was carried out. In addition, histochemical and electron

microscopic examinations were conducted. The main findings consisted of slightly decreased cholesterol and phospholipid content as compared to the unaffected controls but lipid galactose, glucose and galactosamine were moderately elevated. $G_{M1}$-ganglioside was slightly increased in the Sanfilippo patient while $G_{M2}$ was moderately elevated in the Hurler patient. The cytoplasmic inclusions in the Sanfilippo cerebral cortex, as revealed by EM, were of three types: homogeneous granular bodies, zebra bodies and poorly defined concentric membranous bodies.

4. Mucopolysaccharidosis III occurs in two forms referred to as Sanfilippo A and B. The B variant, which has been studied in patients and in tissue culture, is believed to lack the enzyme α-N-acetyl-glucosaminidase. The enzymatic defect in the Sanfilippo A variant has not been definitely established. A partially purified preparation which corrects Sanfilippo A cells in culture has been reported to exhibit sulfatase activity. We have attempted to develop an assay which could be used in the diagnosis of this syndrome. This requires the synthesis, purification and characterization of a substrate for the enzyme which is presumed to be deficient. We have labelled the mucopolysaccharides of Sanfilippo cells in tissue culture with $^{35}S$-sulfate. The labelled mucopolysaccharide has been purified by salt and solvent extraction, protease digestion and chromatography on Ecteola. The product has been characterized by gel filtration, electrophoresis, enzymatic and acidic hydrolysis. It appears to be predominantly heparan sulfate - the mucopolysaccharide which is excreted in the urine of the Sanfilippo patients. This labelled material is being further purified and will be tested as a substrate for mucopolysaccharide sulfatase in leukocytes of Sanfilippo patients and of controls in an effort to establish the enzymatic defect in this disease. If we can in this way develop an assay for the missing enzyme, it will be possible to study the biochemical pathogenesis of the disease. Efforts to isolate the enzyme for experimental enzyme replacement in patients may then proceed.

Significance to Bio-Medical Research and the Program of the Institute: Simultaneous application of basic and chemical studies to these generally sublethal disorders have provided promising data. The time may be not too distant when successful and practical modification of the course of the disease in various mucopolysaccharidoses will become possible. Also, the information obtained will be helpful in diagnosis and prevention of these devastating hereditary disorders.

Proposed Course of the Project: This project carries much promise and will be continued, while emphasis will be placed on enzymatic aspects of the problems concerned and on the basic interaction between disturbance of mucopolysaccharide and lipids.

Honors and Awards: None

Publications:

Constantopoulos, G., Louie, M. and Dekaban, A.S.: Acid mucopolysaccharides (glycosaminoglycans) in normal human kidneys and in kidneys of patients with mucopolysaccharidosis. *Biochemical Medicine* (in press).

Dekaban, A.S. and Constantopoulos, G.: Mucopolysaccharidoses: relation of elevated CSF mucopolysaccharides to mental retardation. *Arch. Neurol.* (in press).

Dekaban, A.S. and Patton, V.M.: Anatomo-pathologie et étude des lipides dans les mucopolysaccharidoses de type Hurler et Sanfilippo. *Nouv. Presse Med*. 17: 415-417, 1973.

Serial No. NDS(1)-68 DMN/EG 1550
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Myelination in the Central Nervous System.

Previous Serial Number: Same.

Principal Investigator: R. O. Brady, M. D.

Other Investigators: John F. Tallman, Ph.D.; Roy M. Bradley

Cooperating Units: Department of Neurology, University of Pennsylvania School of Medicine.

Man Years:

| | |
|---|---|
| Total: | 0.5 |
| Professional: | 0.3 |
| Other: | 0.2 |

Project Description:

Objectives: To investigate biochemical events related to the formation and maintenance of the myelin sheath of nerves. In particular, to establish the exact enzymological reactions which occur during the initiation of myelin deposition, to determine how the integrity of the myelin sheath is maintained, and to obtain an understanding of the biochemical pathology which occurs in demyelinating and dysmyelinating lesions in the nervous system.

Methods: Procedures are being developed for obtaining myelinating explants of neonatal tissue in culture. The effects of selected enzymes and enzyme inhibitors on the myelination process are being examined.

Major Findings: By altering the activity of certain enzymes through the use of specific inhibitory substances we have produced "myelin figures" in tissue culture which very closely resemble the membranous cytoplasmic bodies which occur in patients with Tay-Sachs disease.

Significance: Our procedures should permit examination of the role and contribution of various types of cells and enzymes for the formation and maintenance of the myelin sheath. This information is of particular importance for investigating biochemical abnormalities in myelinopathic conditions such as multiple sclerosis, hereditary demyelinating diseases, and lipid storage diseases which affect the central nervous system.

Proposed Course: This co-operative investigation has been terminated due to the uncertainties attendant to the reorganization of the Department of Neurology, University of Pennsylvania, School of Medicine. The salient aspects of this project will be subsumed by DMN Project 1808.

Publications:

None.

Serial No. NDS(I)-69 DMNB/ND 1655
1. Developmental and Metabolic Neurology Branch
2. Section on Neurological Development
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of the Composition and Biosynthesis of Cerebral Proteins in Experimental Animals and Man.

Previous Serial Number: Same

Principal Investigator: Dennis F. Cain, Ph.D.

Other Investigators: Anatole S. Dekaban, M.D., and John M. Van Buren, M.D.

Cooperating Units: Surgical Neurology Branch, IR, NINDS

Man Years:

| | |
|---|---|
| Total: | 1.3 |
| Professional: | 1.0 |
| Other: | 0.3 |

Project Description:

Objectives: Brain proteins differ from those of other tissues particularly in the elevated levels of the acidic proteins. They are also rapidly synthesized and degraded - a phenomenon without a functional correlate.

An understanding of the role of these specialized aspects of protein metabolism in normal nervous tissue requires determination of the identity composition and relative rates of synthesis and degradation, as well as the localization of individual proteins within various cell types and subcellular organelles. This information is particularly important in the case of the acidic proteins, some of which are known to be brain-specific. Acidic proteins in other tissues have been shown to exert a regulatory function in RNA and protein metabolism. The possibility of interaction of acidic proteins in nervous tissue with neurotransmitters and neuropharmacological agents is of especial significance in neural tissue.

The application of appropriate extraction and separation procedures will allow the determination of both the spectrum and the variability in the composition of the soluble proteins in normal human brain. Thus, changes in the proteins associated with neurological diseases may be examined. The purpose of such studies is to screen normal and pathological surgical and necropsy specimens for the presence or absence of particular protein fractions which may reflect changes in cell structures and cell populations characteristic of various pathological states.

Methods Employed: Working initially with the laboratory animals, various protein fractions are extracted and purified by salt fractionation, isoelectric precipitation, gel filtration, ion exchange, chromatography, etc. These proteins are subsequently analyzed by electrophoresis and isoelectric focusing in a soluble acrylamide gel system. The specific purified protein fractions are also characterized by amino acid analysis, sedimentation and immunological procedures.

For turnover studies, the brain proteins are labeled *in vivo* by intraventricular injections or *in vitro* (tissue slices) with $^{14}$C- or $^{3}$H-leucine. Specific radioactivities of the individual protein fractions are determined by measurement of both protein and radioactivity after separation in the soluble acrylamide gel system which was devised for this work.

Evolving techniques for the separation of subcellular as well as whole cell units will continue to be evaluated for purposes of localization of the individual protein fractions. Tissues from specific areas of the brain and pathological specimens containing a predominant cell type will be especially valuable for this purpose.

Material: Brains and other organs of various small animal species will be used. In addition, samples of brain and other nervous tissue obtained during surgery and at autopsy will be utilized.

Major Findings:
1. A series of normal and abnormal brain tissue specimens were obtained during surgical procedures, portions of which were evaluated histologically or extracted with dilute buffer. The soluble proteins were separated in polyacrylmaide gels by electrophoresis and isoelectric focusing.

Five pairs of histologically normal gray and white matter specimens showed remarkably little variability in distribution of proteins on electrophoretic separation. Overall, twelve of the patients showed very similar gel electrophoresis patterns ("typical" pattern). This group includes not only all of the histologically normal specimens but, in addition, two cases of juvenile lipidosis, one case of mild gliosis, one mucopolysaccharidosis type III and one patient with Unverricht epilepsy.

When extracts of gray matter were compared with corresponding specimens of white, an increased density of several specific bands was apparent. These fractions separate electrophoretically from albumin but migrate in the same general region of the gels. No differences in the gel patterns were correlatable with the different areas of the cortex from which the various specimens were taken.

Six of the patients showed gel patterns which were obviously different from the "typical" distribution. One of these (a glioblastoma) differed only in the increase in band 9. Another glioblastoma showed a virtual absence of nearly all brain proteins usually found in the gels. Two astrocytomas and one specimen of a surgical scar showed a prominent, dense band (#15) and a novel minor component (band 20) while one case of subacute sclerosing panencephalitis showed only the additional heavy band in the area of band 15. These two bands appear to correlate with the histological observation of a marked increase in astrocytic elements in these three patients. Efforts to purify and characterize these proteins are underway. The protein composition of a number of post-mortem neurological specimens are being studied for comparison with the series of surgical specimens.

2. Our previous studies of the developmental alterations in the composition and metabolism of brain proteins (in correlation with structural maturation) have been extended from the fetal stages into the early postnatal period in rabbits. Electrophoretic and isoelectric separation of the extractable brain proteins show a progressive alteration in the content of several particular acidic brain proteins during the first few weeks of life. One acidic protein especially prominent in the newborn becomes apparent at about 11 days of age at a time when myelin deposition becomes apparent.

Significance to Bio-Medical Research and the Program of the Institute: There is some suggestive and still indirect evidence that specific cerebral proteins may be involved in the processes of memory and intellectual functions. Correlation between stimulatory events and the products of synthesis are lacking. There are large numbers of subjects with undifferentiated type of mental retardation who defy all investigative approaches in clarifying the underlying abnormality. Because of several technical difficulties the brain proteins have not been thus far explored in these and many types of other patients. The availability to us of surgical and post-mortem tissue of a number of hitherto obscure neurological diseases provides us with unique opportunity to study cerebral proteins in these patients.

Proposed Course of the Project: This project will continue through fiscal year 1974.

Honors and Awards: None

Publications:

Cain, D.F., Ball, E.D. and Dekaban, A.S.: Brain proteins: qualitative and quantitative changes, synthesis and degradation during fetal development of the rabbit. J. Neurochem. 19: 2031-2042, 1972.

Serial No. NDS(1)-70 DMN/EG 1808
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Glycoprotein Metabolism in the Myelinating Central Nervous System

Principal Investigator: R. H. Quarles, Ph.D.

Other Investigators: R. O. Brady, M.D., J. L. Everly, Ph.D., M. Druse, Ph.D., and J. M. Matthieu, M.D.

Cooperating Units: Section on Cellular Neuropathology, LNNS, NINDS

Man Years:

| | |
|---|---|
| Total: | 3.8 |
| Professional: | 3.8 |

Project Description:

Objectives: To investigate the biochemistry of the surfaces of cells of the nervous system with particular regard to glycoprotein components and their role in myelination. Also, emphasis will be placed on the role of myelin-associated glycoproteins in demyelinating conditions such as multiple sclerosis.

Methods: Specific radioactive sugar precursors are used to label specifically CNS glycoproteins. Myelin and other subcellular fractions are purified by differential centrifugation on sucrose gradients. The purified myelin is subfractionated into light, intermediate and heavy myelin. Enzyme markers for various subcellular structures are used to characterize different fractions. The membrane-bound proteins and glycoproteins are fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The glycoproteins are detected on gels by determination of radioactivity, and double label isotope techniques are used to reveal subtle differences between fractions. Periodic acid-Schiff staining is used for detecting and quantitating glycoproteins on gels. Fast green staining is used for quantitating other myelin proteins on the gels.

Major Findings: It has been shown that rat brain myelin contains a glycoprotein with an apparent molecular weight of approximately 110,000 daltons. A glycoprotein with similar electrophoretic mobility is also present in bovine myelin and human myelin. In the adult rat the glycoprotein accounts for only about 1% of the total myelin protein. Numerous developmental, regional and other experiments have indicated that the glycoprotein is in myelin or an intimately associated structure. Possible localizations suggested by these experiments include the myelin membrane itself, the oligodendroglial plasma membrane, and the axolemma of myelinated axons.

The glycoprotein and other myelin components were determined in myelin subfractions of different densities. The glycoprotein and other high molecular weight proteins are more concentrated in heavy myelin than in light myelin, whereas the myelin basic protein is most concentrated in light myelin. The increase in glycoprotein in the heavier fractions paralleled closely an increase in 2', 3'-cyclic nucleotide phosphohydrolase, which is a marker for myelin or the oligodendroglial plasma membrane. However, the distribution of acetylcholinesterase, which was determined as a marker for the axolemma was different from the glycoprotein. These results clearly show that myelin is biochemically heterogeneous, and that the glycoprotein is not uniformly distributed in the myelin. Furthermore, the similar distribution of the glycoprotein and cyclic nucleotide phosphohydrolase suggests that the glycoprotein is in myelin or oligodendroglial membranes rather than the axolemma. Electron microscopic studies in collaboration with Dr. Webster of LNNS are underway to attempt to elucidate the morphological basis for the biochemical heterogeneity of myelin and to define more precisely the localization of the glycoprotein.

Sensitive double label procedures have conclusively shown that the newly synthesized myelin-associated glycoprotein in 14-day-old rats has a higher apparent molecular weight than that from more mature rats. This finding suggests that the composition of the glycoprotein is different in immature myelin than in mature myelin and that much of the myelin glycoprotein is initially in a precursor fraction with properties different from myelin.

The "jimpy" and "quaking" murine mutants have been examined to see if the glycoprotein is abnormal in these animals in which myelin formation is prevented or reduced. The major myelin glycoprotein is absent or greatly reduced in purified myelin from the jimpy mice whereas other glycoproteins are increased. In the quaking mice, which myelinate to a greater extent than the jimpy mice, the glycoprotein is present but reduced compared to controls.

Preliminary experiments with myelin from multiple sclerosis brain suggest that the glycoproteins are abnormal, but these experiments must be confirmed and extended.

The glycoprotein in CNS myelin has been partially purified and work toward its complete purification is continuing.

Rat sciatic nerve was examined to see if it contained glycoproteins. It did not contain the same glycoprotein as CNS myelin, but the major protein in the PNS myelin appears to be a glycoprotein.

Significance: There is considerable experimental evidence indicating that cell surface glycoproteins mediate specific cell-cell interactions. Therefore glycoproteins could be of great importance in the developing nervous system as many specific interneuronal connections are formed and also as proper neuronal-glial relationships are established. The glycoprotein which is associated with CNS myelin could be involved in specific interactions between oligodendroglial and axonal membranes or between different layers of myelin membranes. The developmental change in the electrophoretic mobility of the glycoprotein could be a valuable handle for elucidating the nature of contact relationships occurring between membranes during the process of myelination. Similarly, the evidence suggesting a precursor of myelin and the abnormalities in the myelin-associated glycoproteins of murine mutants with arrested myelination provide valuable clues about the mechanism of myelin formation. Determination of the precise biochemical mechanisms underlying these phenomena will substantially increase our understanding of myelination.

Two of the leading theories about the nature of multiple sclerosis are that it is caused by an autoimmune response or an infectious agent. Since glycoproteins are known to be cell surface antigens and viral receptors it seems likely that alterations of the myelin-associated glycoprotein might be directly involved in this disease. For example, a virally induced alteration in the glycoprotein could cause it to become the target of an autoimmune response.

Proposed Course: Emphasis will be placed on the purification and chemical characterization of glycoprotein. This is essential in order to biochemically define the role that the glycoprotein plays in myelination and demyelination. Also, subcellular fractionation will continue in order to more precisely define the localization of the glycoprotein in myelin and myelin-associated membranes. This work will be carried out in collaboration with the Section on Cellular Neuropathology so that fractions can be morphologically characterized in the electron microscope. Autoradiography and cell surface probes will also be used to localize the glycoprotein.

Developmental and metabolic studies will continue. Once a procedure for purifying the glycoprotein is available, it should be possible to determine exactly what is responsible for the developmental change in electrophoretic mobility. Efforts will be made to isolate and characterize the myelin precursor whose existence has been suggested by metabolic experiments.

The glycoprotein in multiple sclerosis brain will be examined to see if there are alterations in its amount or carbohydrate composition. The serum from multiple sclerosis patients will be examined to see if it contains antibodies directed against the glycoprotein.

Publications:

1. Quarles, R. H., Everly, J. L., and Brady, R. O.: Demonstration of a Glycoprotein Which is Associated with a Purified Myelin Fraction From Rat Brain. Biochem. Biophys. Res. Commun. 47: 491-497, 1972
2. Glasgow, M. S., Quarles, R. H., and Grollman, S.: Metabolism of fucoglycoproteins in developing rat brain. Brain Res. 42: 129-137, 1972.
3. Everly, J. L., Brady, R. O., and Quarles, R. H.: Evidence that the Major Protein in Rat Sciatic Nerve Myelin is a Glycoprotein, J. Neurochem. in press.
4. Glasgow, M. S. and Quarles, R. H.: Appearance of [$^3$H]water in rat tissues resulting from the metabolism L-[$^3$H]fucose by intestinal bacteria, Biochim. Biophys. Acta, in press.

Serial No. NDS(I)-73 DMNB/ND 2024(c)
1. Developmental and Metabolic Neurology Branch
2. Section on Neurological Development
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Study of Plasma Partitioned Lipids, Lipoprotein and Blood Viscosity in Patients with Transient or Definitive Strokes and in Family Members with Hyperlipidemia.

Previous Serial Number: None - New Project

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Jan K. Steusing

Cooperating Units: In negotiating phase.

Man Years:

Total: (new project)
Professional:
Other:

Project Description:

Objectives: Cerebrovascular disease is one of the most common causes of neurological morbidity and mortality among senior citizens; the condition also occurs with a substantial frequency during middle adult years and low frequency under 20 years of age. Atherosclerotic plaques are the most common pathological lesions encountered in the occluded arteries. Of various factors predisposing to atherosclerosis is persistent elevation of plasma lipids (whether exogenous - dietary, or endogenous - hereditary hyperlipidemia) and abnormal patterns of lipoproteins. It is our intention to study the content and composition of partitioned lipids and lipoproteins in patients with transient or definitive strokes and in persons at high risk such as members of families with hyperlipidemia. Special dietary measures will be made available to these patients and arrangements will be made for their follow-up.

Material: Patients with transient cerebral ischemic attacks or definitive strokes; patients and their families with genetic hyperlipidemias.

Methods Employed:

1. Detailed personal and family medical history including dietary habits.
2. General medical and neurological examinations.

3. Special examination of the retinal blood vessels and peripheral arteries.
4. Radiological, electroencephalographic, electrocardiographic, blood viscosity studies and usual routine laboratory surveys of blood, urine and CSF.
5. Special tests will include - when indicated for diagnostic or planned therapeutic reasons - cerebral angiography and brain scan.
6. Determination of plasma partitioned lipids using established chemical procedures as employed by us in studies of high fat diet in children administered for treatment of epilepsy. Determination of plasma lipoproteins by unidimentional paper electrophoresis supplemented by polyacrylamide gel electrophoresis and density gradient centrifugation; the last procedure will be used only in special instances.
7. Long range management and need for follow-up will be discussed with the patients and appropriate arrangements made.

Major Findings: This is a new project.

Significance to Bio-Medical Research and the Program of the Institute: There is an obvious need for additional research on cerebrovascular disease. Since the relationship between the severity of atherosclerosis and cerebral symptoms is reasonably well established, it will be fruitful to evaluate the handling of high fat diet ingested by the subjects with marked differences in fat metabolism. This could lead to the dietary modifications during early life, and may provide one mechanism for reducing the incidence of cerebrovascular damage.

Proposed Course of the Project: This is a new project.

Honors and Awards: None

Publications: None (new project).

ANNUAL REPORT
July 1, 1972 through June 30, 1973
Laboratory of Neuropathology and Neuroanatomical Sciences, IR
National Institute of Neurological Diseases and Stroke

Igor Klatzo, Chief

The past year has brought several new developments in the Laboratory of Neuropathology and Neuroanatomical Sciences. The most important development is related to the progress in defining and solidifying the profile of the Laboratory, especially with regard to relevance of our research to clinical problems in neurological disease. Although the evaluation of the basic reactions of the nervous system to injury and disease remains the prevailing theme of the Laboratory's research, several areas can be identified where intense, collaborative effort has been concentrated. The area of greatest effort pertains to elucidation of pathologic mechanisms in cerebral stroke. To emphasize our concern with regard to stroke and related problems, the previous Section on Comparative Neuropathology has been renamed the Section on Cerebrovascular Pathology. This Section is serving as the coordinating center for collaborative research on stroke involving other sections of the Laboratory.

With regard to pathogenic mechanisms, it can be stated that every stroke is associated with disturbance of cerebral circulation and particularly with cerebral ischemia. As the latter condition constitutes a most important pathologic change in stroke, it has been studied in a coordinated effort by several sections of our Laboratory. Most of these investigations have been carried out on Mongolian gerbils (Meriones unguiculatus) in which, due to frequent incompleteness of the circle of Willis, cerebral ischemia can be produced (in ± 30% of animals) by ligation of a single carotid artery. Our main objective has been to correlate various parameters of vascular and brain tissue responses with a permanent or temporary obstruction of blood supply in order to elucidate basic pathomechanisms of ischemic brain damage. To ascertain the earliest morphological changes in ischemia, which affect mostly the neurons, a systematic study is being undertaken on specificity of nerve cell alteration in acutely infarcted brain tissue due to vascular occlusion. Observations on terminal carbon black perfusions via the left ventricle of the heart have demonstrated filling defects after release of carotid obstruction, i.e., "no reflow" phenomenon. It has been possible to correlate in time this phenomenon with considerable drop in systolic blood pressure which invariably follows release of carotid obstruction. Our studies revealed that "no reflow" can be greatly reduced or prevented by preceding administration of epinephrine or by raising $pO_2$ in the blood. It is obvious that understanding and control of the "no reflow" phenomenon may have some far-reaching clinical implications in the management of stroke patients. Of another potentially great clinical significance are studies of the carbohydrate metabolism in cerebral ischemia. In view of the fact that glucose constitutes the basic nutrient of the brain, supply of which is acutely impaired in ischemia, transport of radiolabeled 2-deoxy-D-glucose from blood to brain was studied in gerbils during cerebral ischemia. It was demonstrated that a significantly increased facilitated transport of glucose occurs in brain subjected to ischemia even in those animals in which carotid ligation did not result in a demonstrable infarction.

In further studies, radioactive glucose uptake was assessed in conditions of hypoxia, hypercapnia, and osmotic blood-brain barrier (BBB) damage. It was shown that a mild hyperosmotic BBB injury stimulated the facilitated transport of glucose from blood to brain. In severe hypoxia and/or hypercapnia, preliminary observations indicated a decrease of the brain uptake for 2-deoxy-D-glucose. Again, the relevance of these studies to clinical conditions is obvious in view of common complications in stroke involving hypoxia and hypercapnia. The other investigations on carbohydrate metabolism in abnormal conditions of the brain have been primarily concerned with various aspects of brain glycogen. The kinetics of brain glycogen in relation to glycogen synthetase and glycogen phosphorylase was studied in brain injury and in cerebral ischemia at various time intervals following an insult. The interrelationships observed were correlated with behavior of cyclic adenosine-3', 5' monophosphate (cAMP). The latter compound, as well as guanosine-3', 5' cyclic monophosphate (cGMP), were studied also in electroshock under normothermic, hyperthermic, and hypothermic conditions. The results of these studies favor the argument that cAMP and cGMP are independently regulated in vivo. Also, they contribute to an increasing amount of evidence favoring association between cAMP and the aminergic system and between cGMP and the cholinergic system. Thus, neural events following electroshock treatment or brain injury might be defined by measuring the cyclic nucleotides.

Another serious complication in stroke, in addition to ischemia, is the development of brain edema. Although this complication is considered to be an important factor in causing mortality, the nature and dynamics of ischemic edema have been largely unknown. Investigations using gerbils revealed that edema, defined as accumulation of abnormal fluid in the brain tissue and measured by wet/dry weight ratios, develops gradually after 3 hours reaching its peak at 18 hours after ischemic insult. Only at this time (18 hours), there is a demonstrable leakage of BBB tracer (Evans Blue). Morphological studies have further supported the assumption that brain edema following ischemia is originally of the cytotoxic type and only in later stages there is also a vasogenic component due to severe vascular injury and leakage of the serum contents into the brain parenchyma. In another study on edema this pathologic alteration was studied in the spinal cord. Preliminary observations have revealed that unlike the brain, the human spinal cord becomes firmer during swelling because of restriction of swelling by the meninges.

There is increasing evidence that biogenic amines, especially serotonin and norepinephrine play an important role in the pathogenesis of brain edema and brain injuries such as stroke. A systematic assay on biogenic amines in our experimental model of cold injury edema demonstrated strikingly high levels of serotonin in the area of the cortical cold injury, whereas below in the edematous white matter, the serotonin levels were normal and those of norepinephrine were reduced (± 75% of the normal). Application of specific histofluorescent microscopy methods revealed blood vessels in the area of cold injury displaying bright yellowish-green fluorescence. In collaboration with the Chairman of the Department of Neurology at the Albert Einstein College of Medicine, N. Y., and using the instrumentation of his department, it was possible to prove by specific spectrofluorometric analysis that the substance responsible for fluorescence of the blood vessels was indeed serotonin. This discovery acquires an additional importance from electron microscopic observations concerning the *effect of biogenic amines on blood-brain barrier to peroxidase*. These studies revealed that serotonin and norepinephrine appear to increase the permeability of arterioles to proteins. It is apparent that the effect of serotonin deposition in injured blood vessels which possibly may occur in a variety of clinically important pathological conditions, can be of great influence in determining course and resolution of brain lesions. Since the extent of brain damage in stroke can be greatly affected by vascular tonus and especially by vascular spasm, biogenic amines may play an important role in this connection. Our preliminary studies reveal that there is a considerable drop in the level of serotonin, dopamine, and norepinephrine in the area of infarction.

Another major research effort concerned with various aspects of *central and peripheral myelin* has been focused more recently on *demyelinative mechanisms involved in multiple sclerosis*. Since the basic pathologic process in this disease involves primarily the myelin sheaths, research relevant to myelin formation, the correlation of membrane structure and chemical composition of myelin, and the use of model systems to study demyelination are of major importance in identifying pathogenetic mechanisms in multiple sclerosis.

Study of optic nerve lesions found in *Xenopus* tadpoles after immersion in *hexachlorophene* has shown that this *model system* can also be of value in *investigating* some types of myelin breakdown in the central nervous system. Within 24 hours, hexachlorophene produced swimming abnormalities in tadpoles. Lesions in their peripheral myelin sheaths were identified *in vivo* and were similar but less severe than those observed in central myelinated fibers after fixation by phase and electron microscopy. In addition, biochemical analyses of subcellular fractions prepared from pooled tadpole brain homogenates showed that the concentration of hexachlorophene (per mg. of protein) was highest in the myelin fraction. The possible *usefulness of this simple model system* for testing substances (antibodies, sensitized cells, etc.) thought to be associated with the *demyelination that occurs in multiple sclerosis* is currently being investigated. As development of peripheral myelin depends on *axon-Schwann cell interactions*, the dynamic behavior of these cells was studied *in vivo* in *Xenopus* tadpole tailfins using the Nomarski differential interference-contrast microscope and after fixation by electron microscopy. Observations relating to surface interactions between axons and Schwann cells and the changes in axon-Schwann cell relationships that occur during mitosis provide a useful framework for considering the geometrically more complex process of axon-oligodendrocyte interaction before and during myelin formation in the central nervous system (CNS). To achieve a better understanding of the morphological and biochemical events accompanying the demyelination that occurs in multiple sclerosis, the electron microscopic appearance of CNS myelin in adult optic nerves is being compared with that of myelin fractions which have been isolated and characterized biochemically. Following aldehyde immersion, optic nerves are being post-fixed in a solution of osmium tetroxide and potassium ferrocyanide before dehydration and embedding. The preliminary findings have indicated that the lamellar structure of myelin is better preserved than it is when conventional fixation is used. Also, electron microscopic differences have been identified in an initial survey of myelin fractions that differ in chemical composition. It is our contention that these studies will contribute to better understanding of pathologic myelin changes in multiple sclerosis.

The third concentrated research area of the Laboratory has been directed toward the mechanisms of regeneration in the central nervous system which has a direct relevance to the problems of human paraplegia. One major development here has been the demonstration that neurons will survive transplantation, provided the immune response is minimized by means of autografting or by avoiding major genetic histocompatibility differences. This finding suggests that the neuron is not nearly so sensitive to anoxia as had heretofore been thought. Furthermore, the surviving transplanted neurons are capable of regenerating their severed processes and establishing a functional relationship with other tissues. In another approach to the problem, an improved histochemical technique for the demonstration of choline acetyltransferse has been developed. This technique is being used to study the influence of one nerve cell upon another in the central nervous system and to study the process of collateral nerve regeneration after selective lesions of the CNS. In studies on neurotrophic regulation, it has been established that the nerve does not merely regulate muscle contraction, but by a "trophic" function, it also initiates and controls molecular modifications in the muscle cell. The recognition of the fact that the nerve is partly responsible for the regulation of gene expression in the muscle cell offers the possibility to study the molecular basis of neuromuscular disorders. In another series of investigations on regeneration within the CNS, which are relevant to problems of human paraplegia, an attempt is being made to determine how to prevent regenerating neurites from establishing improper connections while at the same time permitting them to establish functionally appropriate ones.

A number of individual research projects has been pursued to elucidate some basic aspects of brain function. Structural basis of synaptic transmission has been studied in frog and crayfish using horseradish peroxidase as electron microscopically demonstrable tracer. In observations on transmitter release at the neuromuscular synapse in frog, it was possible to trace directly the recycling of membrane from coated vesicles to synaptic vesicles and back to surface membrane concomitant with transmitter release. Examination of the inhibitory synapses on crayfish stretch receptor cells revealed that these synapses receive synaptic input from other sources. This work contributes to an understanding of the role of membranes in the storage and release of transmitter at synapses and shows that the mechanism for lease of neurotransmitters is similar to that employed for release of andular secretions.

In a long-ranged project to study *permeability relationships of* *rious cellular barriers* within the nervous system, special attention s been given this year to *arachnoid villae* where the cellular arachnoid rrier layer becomes discontinuous at its apposition with the endothelium of e superior sagital sinus. Study of sinus endothelium utilizing tilt roscopy and serial sections has revealed a system of minute pores which e responsible for bulb drainage of cerebrospinal fluid (CSF). This find- g may be of major importance in understanding basic CSF dynamics and ysiology. The studies on *gap junctions*, which are essential for exchange substances between cells, have succeeded in establishing an upper limit the molecular weight of substances that can cross from one cell to another. is work is important for understanding most basic aspects of transport d exchange of substances in the brain tissue.

Serial No. NDS(I)-71 LNNS/CNC 1883
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Allosteric control of fructose-1,6-diphosphatase

Previous Serial Number: SAME

Principal Investigator: Janet V. Passonneau, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0 |
| Professional: | 0 |
| Other: | 0 |

Project Description:

Objectives: To investigate the allosteric properties of fructose-1,6-diphosphatase in red and white muscle and brain.

Methods Employed: Crude muscle or brain homogenates are analyzed for fructose-1,6-diphosphatase activity by a rapid fluorometric procedure, employing auxiliary enzymes coupled to a pyridine nucleotide system.

Major Findings: The low activity of muscle fructose-1,6-diphosphatase can be modified by allosteric control. As found with the liver enzyme adenosine monophosphate is a potent inhibitor. In addition high substrate inhibits the activity of physiological pH. This inhibition can be overcome by the addition of allosteric effectors, which are present in the tissues.

Significance: The relief of inhibition of fructose-1,6-diphosphatase activity by compounds which accumulate in fatigued muscle or hypoxic brain permits the gluconeogenic mechanism to operate and thus conserve metabolic reserves.

Proposed Course of Project: Discontinue for lack of technical help.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/CNC 1884
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

| | |
|---|---|
| Project Title: | Brain Glycogen Synthetase |
| Previous Serial Number: | SAME |
| Principal Investigator: | Janet V. Passonneau, Ph.D. |
| Other Investigators: | Joan P. Schwartz, Ph.D. |
| Cooperating Units: | None |

Man Years:

| | |
|---|---|
| Total: | 0.5 |
| Professional: | 0.5 |
| Other: | 0 |

Project Description:

Objectives: To determine the role of glycogen synthetase in glycogen formation in brain in normal and experimental states.

Methods Employed: Two assay methods have been developed to measure glycogen synthetase. Because the activity is so low in brain, very sensitive analytical procedures are required. The first procedure is a modification of a published method. The substrate, uridine diphosphoglucose, is treated to remove contaminating uridine diphosphate. The brain is incubated with uridine diphosphoglucose for the appropriate time and one of the products, uridine diphosphate, is measured by an enzymatic assay system coupled to reduced diphosphopyridine nucleotide. The second procedure is based on the measurement of $^{14}$C-labelled glycogen formed when the enzyme is incubated with $^{14}$C-uridine diphosphoglucose. The latter method has been adapted from a published technique and modified to increase reproducibility and sensibility.

A third method has been developed which permits a rapid direct one-step analysis of glycogen synthetase. Column chromatography and rapid concentration techniques have been used to purify the enzyme from brain.

Major Findings: Glycogen synthetase I and glycogen synthetase D were both purified 200-fold from pig brain. The D form was prepared by incubating the crude homogenate in the presence of ATP. The native protein kinase had sufficient activity to phosphorylate the I form and the D form was recovered about 90% pure. Both forms after purification were found to require preincubation with glycogen to ensure maximal activity. This is a new observation of great interest to understand the mechanism of glycogen formation. After preincubation, the enzyme-glycogen complex is virtually irreversible and the enzyme is fully active in the absence of additional primer.

Significance: The glycogen synthetase activity is dependent on the form in which it exists as well as the concentration of substrates and modifiers. The regulation of the activity in brain is of great importance to understand the accumulation of glycogen in anesthesia and trauma, and its depletion in early trauma and ischemia.

Proposed Course of Project: The molar ratio of enzyme to substrate will be examined using the purified enzyme and glycogen of differing molecular weights. The number of enzyme molecules bound to the glycogen molecules, as well as the efficacy of the different glycogens to serve as primers will be examined. A portion of this work was presented at the Neurosciences Meeting in Houston, Texas, in October, 1972.

Honors and Awards: None

Publications:

Rottenberg, D. A., Passonneau, J. V., and Lust, W. D.: Glycogen-mediated activation of pig brain glycogen synthetase I and D. Biochem. Biophys. Res. Commun. 48: 1192-1198, 1972.

Passonneau, J. V., and Rottenberg, D. A.: An assessment of methods for measurement of glycogen synthetase activity including a new direct one-step assay. Anal. Biochem. 51: 528-541, 1973.

Serial No. NDS(I)-71 LNNS/CNC 1885
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The kinetics of brain glycogen

Previous Serial Number: SAME

Principal Investigator: Janet V. Passonneau, Ph.D.

Other Investigators:
Hiroshi Watanabe, M.D.
David Lust, Ph.D.
T.-C. Chen, Ph.D.
Vivian Lauderdale, M.S.

Cooperating Units: Office of Biometry, NINDS

Man Years:

| | |
|---|---|
| Total: | 1.7 |
| Professional: | 1.4 |
| Other: | 0.3 |

Project Description:

Objectives: To determine the rate of glycogen deposition and turnover in brain under normal and experimental conditions. To differentiate between the turnover of the outer tiers and limit dextrin of brain glycogen.

Methods Employed: Brain glycogen will be measured employing enzymatic degradation techniques. Total glycogen will be analyzed using phosphorylase *a*, amylo-1,6-glucosidase, oligo-1,4 → 1,4, glucan transferase, phosphoglucomutase, and glucose-6-phosphate dehydrogenase. The outer tiers will be measured using 6 times recrystallized phosphorylase *a* free of debranching complex. The turnover of glycogen will be measured by injecting mice with UL $^{14}C$-glucose and counting the labelled glycogen isolated by a KOH-ethanol extraction procedure. The differential turnover of the outer tiers and limit dextrin will be determined by determining the specific activity of one portion of the isolated glycogen sample without further treatment (= total glycogen), and the specific activity of the sample after treatment with crystalline phosphorylase (= limit dextrin). Agents affecting metabolism of the brain will be used to elucidate the control of glycogen metabolism. Phenobarbital, which depresses the brain metabolism, and hydrocortisone which increases the metabolic rate will be studied.

Major Findings: In addition to the findings reported last year, glycogen synthetase which is the enzyme which makes glycogen was studied. This enzyme exists in two forms, I, which is independent of glucose-6-P for activity, and D, which is inactive in the absence of glucose-6-P. The proportion of the enzyme which exists in these two states is considered to reflect the rate at which glycogen can be synthesized. It was found that the ratio of these two forms did not change either during phenobarbital anesthesia or after administration of hydrocortisone.

A system for the calculation of glycogen turnover was devised with the help of Dr. T.-C. Chen of the Office of Biometry. A 2-compartment system consisting of blood glucose and brain glycogen was proposed assuming (1) that the fraction of glucose going from blood to brain glycogen per unit time is the same for labelled and unlabelled glucose; (2) that the fractional amount of unlabelled glucose incorporated into brain glycogen is independent of time; and (3) the system is in a steady state. The rate constant for incorporation of $^{14}$C-glucose into brain glycogen for control and phenobarbital-treated animals was 2.26 and 2.24 respectively, indicating that anesthesia had no effect on the synthetic pathway. The same rate constant for the hydrocortisone-treated animals was 3.39, significantly higher than controls. On the other hand, the first order rate constant for the disappearance of glycogen was 3.42 for the control animals and was decreased to 1.98 by anesthesia. It is clear that the degradative pathway was affected, accounting for the accumulation of brain glycogen during anesthesia. Following hydrocortisone treatment, the rate constant for the disappearance of glycogen was 7.56, which agrees well with the accelerated turnover of glycogen following hydrocortisone administration. Similar results were obtained when the model was applied to limit dextrin, the inner core of glycogen. The turnover time of the limit dextrin, however, was about twice that of total glycogen.

The effects of trauma on brain glycogen and related metabolites is being investigated. After a cerebral stab wound there is an initial fall in glycogen (10 and 30 min) followed by an increased level of glycogen at 1.5 hours following trauma which persists for a 24-hour period.

Glycogen synthetase I activity is increased following injury at 1 and 10 min. Glycogen phosphorylase a is also increased at these times. Glucose levels decrease 1 min after injury and subsequently increase over control levels and are twice that of controls at 24 hours. Similarly, glucose-6-P is initially decreased, then increased over control levels. The pattern is one of initial ischemia in which energy reserves fall, followed by apparent return of circulation with compensatory increase in the stab wound area. Cyclic adenosine monophosphate levels increase and apparently stimulate the conversion of the phosphorylase and synthetase from one form to the other, mediated by protein kinase and phosphorylase b kinase.

Of particular interest is the effect of trauma on the energy reserves and metabolic rate of brain. The energy reserve is calculated as the sum of

P-creatine, adenosine triphosphate, glucose and glycogen. The metabolic rate is calculated from the sum of the changes in these compounds in 10 seconds following decapitation and expressed as high-energy phosphate equivalents ($\sim$ P) per kg of brain per minute. The following table shows the changes due to stab-wound injury.

| | | $\sim$ P/kg/min |
|---|---|---|
| Control | | 21.1 |
| Time after injury- | 10 sec | 20.0 |
| | 30 sec | 14.2 |
| | 1 min | 12.0 |
| | 10 min | 37.4 |
| | 60 min | 36.0 |
| | 24 hr | 23.1 |

Significance: Brain glycogen levels have been shown to increase in various traumatic and experimental states. The changes in turnover rate in anesthesia and trauma are of great concern in order to understand the changes in metabolism that occur in pathological states.

Some of these data have been presented at the Neurosciences meeting in Houston, October, 1972.

Further work has been done on the relationship of cyclic adenosine monophosphate (cAMP) levels in brain and the forms of glycogen synthetase I and D and phosphorylase a and b. Epinephrine, for example, elevates cAMP in the brain, which in turn activates protein kinase. The protein kinase converts glycogen synthetase I to D, thus decreasing glycogen synthesis. The protein kinase also activates phosphorylase b kinase which in turn activates phosphorylase, promoting glycogen utilization. Thus during stress energy reserves are mobilized. We have investigated the control of the conversion of these enzymes in rat and mouse brain under various conditions. Phosphorylase a, the active form, is about 20% of total phosphorylase in rat and mouse brain. Glycogen synthetase is 21% in the I(active) form. In hypothermic mice the phosphorylase a was 23% of total and synthetase I 48% of total. Cyclic AMP was decreased to 65% of control levels, showing a striking correlation with the enzyme forms. When hypothermic animals are made anoxic, glycogen synthetase I decreased to 23%, phosphorylase a increased to 40% of total, accompanied by a 120% increase in cyclic AMP. Part of this work will be presented at the Federation meetings in Atlantic City by Dr. Lust.

Another aspect of glycogen metabolism was investigated studying the effects of ethanol administration in rats, acute (8 min) and prolonged (13 h) exposures. The effect of acute (8 min) and prolonged (13 h) exposures to high doses of ethanol upon the intermediary metabolites of rat brain has been studied, with the use of a new freezing technique which minimizes post-mortem changes. Injection of ethanol (80 mmol/kg body wt) produced general anesthesia within 8 min after administration. At this time there were increases in the brain contents of glucose, glucose-6-phosphate and citrate; there was no change in arterial $pCO_2$. Rats under ethanol anesthesia for 13 h showed increases in brain contents of glycogen, glucose and glucose-6-phosphate; and decreases in lactate, pyruvate, α-oxoglutarate and malate. Under similar experimental conditions, arterial $pCO_2$ increased from 37 to 51 Torr. The changes in levels of metabolites after injection of ethanol were similar to those after administration of many volatile anesthetic agents or elevation of brain $CO_2$ by other means. Although brain levels of malate and α-oxoglutarate decreased after prolonged exposure to ethanol, the mitochondrial redox state was maintained. Accordingly, the levels of glutamate and aspartate fell in accordance with the law of mass action. The maintenance of the cytoplasmic and mitochondrial redox states in the brain during ethanol intoxication was in marked contrast to the effects on the liver. We suggest that the different effects observed in brain and liver result from the action of ethanol upon the nerve cell membrane in brain, whereas the primary target in liver is alcohol dehydrogenase.

Proposed Course of Project: Further studies are being made on the effect of electro convulsive shock, insulin, and experimental brain edema on the rate of incorporation of $^{14}C$-glucose into brain glycogen and the subsequent loss of label.

Honors and Awards: None

Publications:

Watanabe, H., and Passonneau, J. V.: Factors affecting the turnover of cerebral glycogen and limit dextrin *in vivo*. *J. Neurochem*. 20: 1973, in press.

Veloso, D., Passonneau, J. V., and Veech, R. L.: The effects of intoxicating doses of ethanol upon intermediary metabolism in rat brain. *J. Neurochem*. 9: 2679-2686, 1972.

Serial No. NDS(I)-72 LNNS/CNC 1942
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The role of adenosine 3',5' cyclic monophosphate (cyclic AMP) and guanosine 3',5' cyclic monophosphate (cyclic GMP) in the central nervous system.

Previous Serial Number: SAME

Principal Investigator: W. David Lust, Ph.D.

Other Investigators: Janet V. Passonneau, Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.6 |
| Professional: | 0.6 |
| Other: | 0 |

Project Description:

Objectives: To determine if either pharmacological or physiological alterations of brain metabolism would affect the steady state levels of cyclic AMP and/or cyclic GMP *in vivo*.

Methods Employed: Mice were rapidly frozen in liquid nitrogen at the appropriate times following treatment. The brains were removed at -25°, weighed and extracted in perchloric acid. The neutralized PCA extracts were used in all subsequent metabolic measurements. Cyclic GMP was measured by an enzymic-cycling technique and cyclic AMP was measured by a radioactive binding assay.

Electroconvulsive shock (ECS) was applied by corneal electrodes at an intensite of 50 mA for a duration of 0.2 sec. The electroshock produces a convulsive response manifested by a) a tonic extensor phase (0-15 sec), b) an intermittent clonic phase (15-30 sec) and c) a postictal depressive phase (> 30 sec).

In the thermal experiments, the mice were rendered a) hypothermic (20°) by forcing them to swim for 1-2 min in a water bath followed by a 10 min stay in a cold chamber, and b) hyperthermic (41°) by placing them under a heat lamp. Rectal temperatures were monitored with a tele-thermometer.

Major Findings: The continuation of the work on electroconvulsive shock (ECS) and ischemia (produced by decapitation) confirmed our previous findings that the levels of cyclic AMP increase rapidly following both treatments in all areas of the brain tested. Although the concentration of cyclic GMP does not significantly change during the 60 seconds following decapitation, this cyclophosphate is elevated after ECS in both the cerebellum and the forebrain. The major difference between the cyclic nucleotide responses is that the maximal change in cyclic AMP occurs at 30 seconds and precedes the cyclic GMP peak by approximately 30 seconds. Relating these responses to convulsive behavior, it is apparent that the major cyclic AMP changes occur during the excitable states (tonic, 0-15 seconds and clonic, 15-30 seconds) and the cyclic GMP during the depressive stage (postictal, greater than 30 seconds). Although the levels of cyclic nucleotides drop rapidly following their maximal ECS-induced changes, the restoration of cyclic AMP and cyclic GMP to control levels was not reached by 240 seconds.

To determine what effect altered body temperature would have on cyclic nucleotides, mice were rendered hypothermic (20°) or hyperthermic (41°) as described in the methods. In hypothermic mice, the levels of cyclic AMP were lower than normothermic values in both the cerebellum and the cerebral cortex. The levels of cyclic GMP were not affected. Further, the cyclic AMP increases following either decapitation or ECS were significantly reduced. In hyperthermic mice, both cyclic nucleotides were unchanged in NIH mice; however, the concentrations of cyclic GMP increased 2-fold in the DBA-2N strain of mice.

In pharmacological studies, phenobarbital, papaverine and $MgSO_4$ significantly decreased the levels of cerebellar cyclic GMP. Phenobarbital (100 mg/kg IP) lowered cyclic GMP within five minutes and the levels remained at 20% of control for up to 90 minutes. Of all the drugs tested, only chlorpromazine and trifluopromazine lowered cyclic AMP levels. Pretreatment with two drugs, amphetamine (10 mg/kg IP) which elevates GMP 2-fold, or phenobarbital (20 mg/kg IP) which decreases cyclic GMP 50%, had no effect on the ECS-induced increase in cyclic nucleotides, in spite of significant alterations in overt convulsive behavior.

Using 4 different fixation techniques, the levels of cyclic AMP were lowest in the microwave irradiated brain with increasing amounts in brains which were freeze-blown, frozen by immersion and frozen following decapitation. In comparison, the degree of anoxia as determined by the changes in ATP, phosphocreatine and lactate was lowest in the freeze-blown brain, with increasing anoxia in brains which were frozen by immersion, irradiated, and frozen following decapitation.

Significance: Both the ECS and pharmacological data favor the argument that cyclic AMP and cyclic GMP are independently regulated *in vivo*. The possibility of a direct relationship between elevated cyclic GMP and depression seems remote, since pharmacologically-induced depression either reduced or had no effect on cyclic GMP levels. There is an increasing amount of evidence favoring an association between cyclic AMP and the aminergic system and between cyclic GMP and the cholinergic system. Thus, neural events following such treatments as ECS or brain injury might be defined by measuring the cyclic nucleotides. For example, the aminergic system would predominate in the first thirty seconds after ECS and the cholinergic system from thirty seconds on.

Since cyclic AMP increases rapidly in the brain following decapitation, low cyclic AMP levels have been equated with rapid fixation. However, following microwave irradiation low levels of cyclic AMP were obtained although the irradiated brains were severely anoxic by other criteria. Of the fixation methods tested, freeze-blowing and freezing intact most closely approximate the in vivo state.

Proposed Course of Project: More extensive experiments on the ECS-dependent rise of cyclic nucleotides are being undertaken in other areas of the brain. Using the appropriate pharmacological antagonists, the cyclic nucleotide-putative neurotransmitter relationship after ECS may be elaborated. Further, the structure activity relationship of the phenobarbital-induced decrease in cyclic GMP is being investigated.

Honors and Awards: None

Publications:

Lust, W. D., Passonneau, J. V., and Veech, R. L.: Cyclic adenosine monophosphate, metabolites and phosphorylase in neural tissue: a comparison of methods of fixation. Science, 1973, in press.

Serial No. NDS(I)-73 LNNS/CNC 2006
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Regulation of metabolism in glioma and neuroblastoma cell lines

Previous Serial Number: NONE

Principal Investigator: Joan P. Schwartz, Ph.D.

Other Investigators:
W. D. Lust, Ph.D.
J. V. Passonneau, Ph.D.
V. Lauderdale, M.S.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.0 |
| Professional: | 0.8 |
| Other: | 0.2 |

Project Description:

Objectives: To investigate the regulation of metabolism in a glioma and a neuroblastoma cell line.

Methods Employed: The cells are being grown in a $CO_2$ incubator in the laboratory. Homogenates of the cells are analyzed for various metabolites, such as cyclic AMP, cyclic GMP, glycogen and pyridine nucleotides, as well as for enzymes such as the adenylate cyclase, cAMP phosphodiesterase and glycogen synthetase and phosphorylase. All of these analytical methods have been used in the laboratory on whole brain and are thus easily adaptable for use with the cells. In addition, the effect of various agents on the morphology of the cells will be undertaken when a phase microscope becomes available.

Major Findings: The glioma cells contain an adenylate cyclase which is responsive to catecholamines such as norepinephrine. Activation of the cyclase by norepinephrine elevates the intracellular cyclic AMP level, resulting in the breakdown of glycogen due to inactivation of the glycogen synthetase and activation of the phosphorylase (Browning, E.T., Schwartz, J.P., Shaw, S.H., and Breckenridge, B.M.: Prov. V Int. Cong. Pharm., 1972). The expression of the norepinephrine receptor has been shown to require cell contact. Activity of the cyclic AMP phosphodiesterase can also be regulated, by growth of the cells in either dibutyryl cAMP or in bromo-deoxy-uridine, an agent which causes dedifferentiation (Schwartz, J.P., Morris, N.R. and Breckenridge, B.M.: J. Biol. Chem., in press, 1973).

Significance: A study of the regulation of metabolism in the brain is complicated by the presence of several cell types and the inability to determine in which cells metabolic alterations are occurring. Such studies are facilitated by the use of the two distinct cell lines, the glioma (rat C6 line) and the neuroblastoma (mouse C1300 line), because regulation can be studied in each cell line separately. Until these cell lines became available, for example it had not been realized that glial cells might be hormone-responsive. Such results suggest that the glial cells may have a greater involvement in brain function than the supportive one originally envisaged. Although these two cell lines are tumor cells, they offer a first approach to the problem of regulation of metabolism in the brain, which might be followed up in normal brain cells when the isolation techniques for glial and neuronal cells have been perfected.

Proposed Course of Project: A thorough investigation of the regulation of cyclic AMP and cyclic GMP levels, of glycogen metabolism and other energy-related parameters, and of pyridine nucleotide ratios will be undertaken, in both cell lines. Studies on ion transport are also planned, as are morphological studies of the effects of various agents and growth conditions.

| | |
|---|---|
| Honors and Awards: | None |
| Publications: | None |

Serial No. NDS(I)-73 LNNS/CNC 2007
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

| | |
|---|---|
| Project Title: | Regulation of the $NAD^+/NADH$ ratio in normal and virally-transformed tumor cell lines |
| Previous Serial Number: | NONE |
| Principal Investigator: | Joan P. Schwartz, Ph.D. |
| Other Investigators: | Janet V. Passonneau, Ph.D.<br>George S. Johnson, Ph.D.<br>Ira Pastan, M.D. |
| Cooperating Units: | Laboratory of Molecular Biology, NCI |

Man Years:

| | |
|---|---|
| Total: | 0.5 |
| Professional: | 0.5 |
| Other: | 0 |

Project Description:

Objectives: To investigate factors which regulate the ratio of total and free $NAD^+/NADH$ in normal and transformed cell lines.

Methods Employed: The cells are homogenized following growth under various conditions. The homogenates are analyzed for $NAD^+$ and NADH by a fluorometric procedure which involves enzymatic cycling to provide the necessary sensitivity.

Major Findings: The total $NAD^+/NADH$ ratio is low (2-3) in log phase normal cells but rises with confluency (to 10-12). However, no such rise in the ratio is seen in the transformed tumor cells as they become confluent. Corresponding changes were found in the ratio of the free pyridine nucleotides. These changes could not be correlated with intracellular cyclic AMP levels. Most of this work has been carried out with mouse 3T3 fibroblasts and their SV40-virus transformed tumor cell counterpart. However, the results have been confirmed in 2 other cell lines, the NRK (normal rat kidney) cell line before and after transformation, and the L-929 line (mouse connective tissue cells).

Significance: The pyridine nucleotides ($NAD^+$ and NADH) are involved in many of the oxidation-reduction reactions of a cell. The ratio of $NAD^+$ to NADH is therefore a basic indicator of the metabolic state and growth properties of the cells. The differences in the ratio found between normal and tumor cells must therefore reflect the metabolic alteration in transformed cells. The ability to regulate this ratio in confluent transformed cells might thereby allow restoration to the growth and metabolic state of normal cells.

Proposed Course of Project: Various growth-altering compounds, such as serum, trypsin and insulin, are now being investigated for their effect on the $NAD^+$/NADH ratio.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70 LNNS/CN 1802
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Study of living nerve and muscle fibers by differential interference microscopy

Previous Serial Number: Same

Principal Investigator: Susan M. Billings, Ph.D.

Other Investigators: Henry deF. Webster, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.45 |
| Professional: | 0.3 |
| Other: | 0.15 |

Project Description:

Objectives: To study migratory Schwann cells during development and characterize their structure, movements, and relationships to axons or other cell processes.

Methods Employed: Peripheral nerve fibers in the *Xenopus* tadpole tail-fin have been examined both *in vivo* with the Nomarski differential interference-contrast microscope and after fixation by electron microscopy.

Major Findings: Several different types of migratory cells are closely associated with developing axons. These include fibroblasts and white blood cells as well as Schwann cells. The *in vivo* appearance and behavior of these different cells have been documented and their fine structure has been described. Schwann cells generally migrate along nerve fibers, but they are capable of leaving the fibers and moving freely for at least short distances. During this migratory phase, the shape of the cell is an ovoid, distorted by several rather long processes that end in thin, blunt expansions. Often one of these is wrapped around an axon or bundle of axons. In the electron microscope, these Schwann cells display long slender processes and highly irregular ruffled borders typical of migrating cells. The complement of cytoplasmic organelles is generally similar to that of older sedentary Schwann cells. At this stage, the cell has no basal lamina. Next the Schwann cells settle down, assume their characteristic spindle shape, and become more intimately associated with one or more axons. Later still, they acquire a basal lamina. This may be a morphological sign that the Schwann cell has entered into some long lasting relationship with those fibers it now surrounds.

Significance to Biomedical Research and the Program of the Institute: The correlation of the dynamic behavior of Schwann cells *in vivo* and their electron microscopic appearance during migration and establishment of relationships with axons adds significantly to an understanding of axon-Schwann cell interactions during development. In addition, these observations are an essential prerequisite to pathological studies designed to test the effects of drugs or lesions on this model system.

Proposed Course of Project: A manuscript entitled "Myelinated nerve fibers during development: *In vivo* and electron microscopic observations of *Xenopus* tadpoles" will be submitted to Developmental Biology. Future studies will use this model system to study demyelination (see Serial No. LNNS/CN 1995)

Honors and Awards: None

Publications:

Webster, H. deF., Billings, S. M., and Guth, L.: Muscle fibers and their innervation in *Xenopus* tadpoles: Fine structure, histochemistry, and *in vivo* observations. In Kakulas, B. A. (Ed.): *Myology. Proceedings of the Second International Congress on Muscle Diseases*. Amsterdam, Elsevier, 1973, pp. 549-555.

Serial No. NDS(I)-70 LNNS/CN 1803
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electron microscopic study of axon-Schwann cell relationships prior to myelination in peripheral nerves

Previous Serial Number: Same

Principal Investigator: Henry deF. Webster, M.D.

Other Investigators: John Martin, B.S.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.1 |
| Other: | 0.1 |

Project Description:

Objectives: To define the geometry of mitotic Schwann cells and changes in their relationships with axons during the mitotic cycle.

Methods Employed: Three dimensional reconstructions were prepared from electron micrographs of transversely and longitudinally sectioned sciatic nerves of newborn rats.

Major Findings: During prophase, the radial extent of sheet-like processes surrounding axons was progressively reduced until the Schwann cell was spindle shaped. Numerous 100 nm. vescicles were found in the cytoplasm as the surface area decreased; they disappeared during the subsequent re-extension of the radial processes. The axis of mitosis was parallel to the long axis of the cell and transverse cleavage of the cytoplasm occurred between the daughter nuclei. Radial processes that surrounded axons reappeared in telophase; they began forming in the nuclear region and subsequently grew radially and extended peripherally toward both ends of the cell.

Significance to Biomedical Research and the Program of the Institute: During peripheral nerve development, division increases the number of Schwann cells available for axonal sorting. The location of the dividing cells and the changes in their shape during division probably include surface interactions that are important in the selection of axons to be myelinated. Now that the basic parameters of the changing geometry and axon relationships of dividing Schwann cells have been defined in normal nerves, alterations associated with developmental abnormalities of axon-Schwann cell interaction before myelination can be characterized. In addition, these studies provide a useful framework for considering the geometrically more complex process of axon-oligodendrocyte interaction before and during myelin formation in the CNS.

Proposed Course of Project: To be continued by using radioautography to investigate the axon relationships of Schwann cells actively synthesizing DNA prior to division.

Honors and Awards: None

Publications:

Martin, J. R., and Webster, H. deF.: Mitotic Schwann cells in developing nerve; Their changes in shape, fine structure, and axon relationships. Develop. Biol. (In Press)

Webster, H. deF., Martin, J. R., and O'Connell, M. F.: The relationships between interphase Schwann cells and axons before myelination. Develop. Biol. (In Press)

Webster, H. deF.: Peripheral nerve structure. In Hubbard, J. I. (Ed.): The Vertebrate Peripheral Nervous System. New York, Plenum Press, 1973. (In Press)

Serial No. NDS(I)-73 LNNS/CN 1995
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Morphological studies of experimental demyelination in model systems

Previous Serial Number: None

Principal Investigator: Henry deF. Webster, M.D.

Other Investigators: Andrew G. Ulsamer, Ph.D.

Cooperating Units: Dermal Toxicity Branch, FDA

Man Years:

| | |
|---|---|
| Total: | 1.5 |
| Professional: | 0.8 |
| Other: | 0.7 |

Project Description:

Objectives: (1) To study the appearance and distribution of lesions produced by hexachlorophene (HCP) in myelin and other cellular components of the nervous system in *Xenopus* tadpoles. (2) To measure the HCP content of brains from tadpoles exposed to HCP and determine its distribution in subcellular fractions prepared from pooled brain homogenates.

Methods Employed: *Xenopus* tadpoles were immersed in growing solution containing 0.1 or 0.2 mcg. of HCP per ml. for intervals up to seven days. Myelinated nerve fibers in the tailfin were examined *in vivo* with the Nomarski differential interference-contrast microscope. After fixation, sections containing muscle, peripheral nerve, sensory ganglia, spinal cord, brain, and optic nerves were studied by phase and electron microscopy. The HCP content of tadpoles and their brains was measured by gas liquid chromatography. The subcellular distribution of HCP-$^{14}$C was studied in fractions that were characterized by electron microscopy and analyzed chemically for proteins, phospholipids, and sterols.

Major Findings: Within 8 hours, tadpoles exposed to 0.2 mcg. HCP/ml. usually developed abnormalities in posture and swimming movements that persisted. *In vivo* examination of nerve fibers in the ventral tail region after 24 hours revealed focal splitting and vacuolation in occasional myelin sheaths. In the sections prepared for phase and electron microscopic study, similar lesions were present; they were larger and more numerous in central than in peripheral myelin sheaths. Following immersion in either 0.1 or 0.2 mcg. HCP/ml., the tadpoles absorbed 1.6-1.9 mcg./gm. body weight in the first 24 hours. The levels achieved after three days in the two concentrations were 3.4 and 17.1 mcg./gm. body weight respectively, and similar levels were also observed after immersion for seven days. The brains of tadpoles immersed in 0.2 mcg. HCP/ml. for three days contained 1.3-5.9 mcg. HCP/gm. wet weight. Finally, analyses showed that the myelin fraction's specific radioactivity per mg. protein was approximately twice that in the other fractions (microsomes, nerve endings, mitochondria, nuclei and cell debris).

Significance to Biomedical Research and the Program of the Institute: These results show that HCP can produce rapid changes in the compact lamellar structure of myelin that may be due to direct interaction with its membrane components. Central meylin sheaths may be more susceptible than those in the PNS to the action of HCP because they are not surrounded by a satellite cell sheath; differences in CNS and PNS myelin composition may also determine the distribution and severity of lesions. Finally, these observations emphasize the usefulness of this model system for correlative interdisciplinary studies of both CNS and PNS demyelination.

Proposed Course of Project: A manuscript describing the above findings will be submitted to the Journal of Neuropathology and Experimental Neurology. Future experiments will attempt to use this model system to test substances thought to be important in the demyelination associated with multiple sclerosis and to study remyelination in the CNS.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/CN 1996
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Myelin membrane structure - morphological comparisons in intact tissue and subcellular fractions

Previous Serial Number: None

Principal Investigator: Henry deF. Webster, M.D.

Other Investigators: Richard H. Quarles, Ph.D.

Cooperating Units: Developmental and Metabolic Neurology Branch, NINDS

Man Years:

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.05 |
| Other: | 0.05 |

Project Description:

Objectives: (1) To compare the electron microscopic appearance of CNS myelin in adult optic nerves with that of myelin fractions which have been isolated and characterized biochemically. (2) To carry out similar comparisons during CNS myelin formation. (3) To apply the results to studies of demyelination in multiple sclerosis.

Methods Employed: Following aldehyde immersion, optic nerves have been post-fixed in a solution of osmium tetroxide and potassium ferrocyanide before dehydration and embedding.

Major Findings: Only preliminary observations are available. The lamellar structure of myelin is better preserved than it is when conventional fixation is used. Also, electron microscopic differences have been identified in an initial survey of myelin fractions that differ in chemical composition.

Significance to Biomedical Research and the Program of the Institute: To achieve a better understanding of the morphological and biochemical events accompanying the demyelination that occurs in multiple sclerosis, new methods must be tested in animal CNS myelin before selecting those best suited for studying human myelin and MS tissue.

Proposed Course of Project: to be continued.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-72 LNNS/CP 1964
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Sequelae of osmotic blood-brain barrier (BBB) damage

Previous Serial Number: NDS(I)-72 LNNS/CN 1964

During FY 1973 this project was incorporated with Serial No. NDS(I)-71 LNNS/NC 1880.

Serial No. NDS(I)-73 LNNS/CP 1997
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cerebrovascular reactions to ischemia in the Mongolian gerbil

Previous Serial Number: None

Principal Investigator: Umeo Ito, M.D.

Other Investigators: Igor Klatzo, M.D.
Maria Spatz, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.80 |
| Professional: | 0.50 |
| Other: | 0.30 |

Project Description:

Objectives: The Mongolian gerbil (Meriones unguiculatus) is a rodent which frequently lacks connecting arteries between the basilar and carotid systems. Consequently, occlusion of the common carotid artery results in cerebral infarction in approximately 30% of the animals. For this reason, the Mongolian gerbils offer a unique opportunity of studying the effects of cerebral ischemia in a large series of animals and using a simple operative procedure of applying a removable clip to the common carotid artery. The objective of this study is to correlate various parameters of vascular and brain tissue responses to a permanent or temporary obstruction of blood supply in order to elucidate the pathomechanisms of ischemic brain damage.

Methods Employed: Mongolian gerbils were subjected to unilateral or bilateral carotid ligations for various periods of time. The status of cerebral circulation was assessed by: (1) radioautographic study of the cerebral blood flow (CBF), (2) Pickworth benzidine stain demonstrating red blood corpuscles, and (3) terminal perfusions with suspensions of carbon black inserting a cannula into the left ventricle of the heart. The behavior of the blood-brain barrier (BBB) was studied with Evans Blue tracer. All of the brains were subjected to histological examination.

Major Findings: The investigations demonstrated a reduced CBF in the infarcted areas, whereas the adjacent zones frequently revealed a "luxury perfusion syndrome." In benzidine preparations, dilated blood vessels suggested a passive hyperemia in the area of infarction. The changes in the BBB in the infarcted hemisphere occurred only 18 hours after ligation. Perfusions with carbon black, carried out at various time intervals after release of unilateral or bilateral carotid obstructions, revealed filling defects in some areas of the brain. These filling defects were demonstrable for about 30 minutes after unilateral ligation on the side of infarction and occasionally also on the opposite side. The occurrence of the filling defects could be abolished or reduced by administration of epinephrine before the release of carotid clipping.

Significance to Biomedical Research and the Program of the Institute: These studies are of paramount importance for the understanding of basic pathologic mechanisms in stroke and in cerebral ischemia. Observations with carbon black demonstrating filling defects after release of carotid obstruction are relevant to the "no reflow" phenomenon described in the literature. Understanding and control of this phenomenon may have far reaching clinical implications in the management of stroke patients.

Proposed Course of the Project: Further investigations will be directed to evaluation of various aspects of "no reflow" phenomenon. Effect of various pharmacological agents will be tested as well as the effect of various $pO_2$ and $pCO_2$. Observations on the carbon black filling of the cerebral blood vessels will be correlated with biochemical, histochemical, and electron microscopic studies on the brain tissue.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/CP 1998
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Central biogenic amines in edema and ischemia

Previous Serial Number: None

Principal Investigator: Jonathan Costa, M.D., Ph.D.

Other Investigators: Umeo Ito, M.D.
Igor Klatzo, M.D.
Maria Spatz, M.D.

Cooperating Units: Laboratory of Clinical Science, NIMH

Man Years:

| | |
|---|---|
| Total: | 1.8 |
| Professional: | 1.8 |
| Other: | 0.0 |

Project Description:

Objectives: (1) To determine what role, if any, central biogenic amines (serotonin, dopamine, norepinephrine) play in the pathogenesis of brain edema and ischemia. (2) To examine whether the production and turnover of biogenic amines is affected in brain edema and ischemia.

Methods Employed: (1) Cold-induced edema (Cat). A "cold lesion" is made by applying a cold (-50°C) plate to the exposed cerebral cortex of the cat for 30 seconds. At various times after the lesion, lesion and control tissue is taken for assay of endogenous serotonin, dopamine, and norepinephrine levels, and for fluorescent histochemistry and spectrofluorophotometry. In some experiments, radioactive serotonin or norepinephrine is injected prior to making the lesion. Control and lesion tissue is taken for counting of total and specific activity (activity present as either serotonin or norepinephrine). Platelet serotonin will be labeled with radioactive serotonin, and its entry into and disposition in injured cat cortex will be examined. Following depletion of platelet serotonin with chronic imipramine treatment, levels of serotonin in injured cat cortex will be studied. (2) Ischemia (Gerbil). The carotid arteries are clipped and released in various sequences and for various time intervals. Whole brains are taken for biogenic amine assay and fluorescence histochemistry.

Major Findings: (1) Cold-induced edema (Cat). White matter edematous areas in the cat brain show a 30% drop in endogenous norepinephrine levels, possibly due to an inhibition of norepinephrine synthesis. The area immediately adjacent to the necrotized cortical surface reveals a 400% increase in endogenous serotonin. The blood vessels in this area showed a bright yellowish-green fluorescence, typical for serotonin. This increase in serotonin may represent material released from platelets in the injured area. (2) Ischemia (Gerbil). Animals subjected to unilateral ischemia for six hours show a 100% drop in endogenous levels of serotonin, dopamine, and norepinephrine. Levels of biogenic amines in other pathological conditions are still under study.

Significance to Biomedical Research and the Program of the Institute: Biogenic amines, especially serotonin and norepinephrine, have been recently implicated in the pathomechanisms of brain edema and various forms of brain injury. This is of great potential importance since it has been established that biogenic amines play a considerable role in vasoregulation and in behavioral and psychic dynamics. These aspects can greatly influence clinical condition of a brain edema or cerebral stroke patient. Discovery that the injured cerebral vessels contain high amounts of serotonin, combined with observations from the project NDS(I)-72 LNNS/NC 1965 indicating that serotonin and epinephrine may increase permeability of cerebral vessels, is of great potential significance in evaluation of the role of such blood vessels in dynamics of brain edema or head injury. The elucidation of vasomotor phenomena and their relationship to biogenic amines in cerebral ischemia may significantly contribute toward designing efficient methods of management and treatment for the cerebral stroke victims.

Proposed Course of the Project: This project will be continued, especially with regard to evaluation of the role of biogenic amines with regard to vasoregulation and cerebrovascular permeability in brain edema and ischemia which are the main pathological components of the cerebral stroke.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-62 LNNS/ENP 944
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Hematologic control of primates

Previous Serial Number: SAME

Principal Investigator: Mrs. Margaret G. Johnson

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.0 |
| Professional: | 0.0 |
| Other: | 0.0 |

Project Description:

With the transfer of the principal investigator into the Stride program, this project was discontinued, although the hematologic control of primates represents an essential step in determining the status of health of animals prior to an experiment, because they frequently suffer from severe parasitemia (Plasmodium, Trypanasoma) and deficiency anemia.

Serial No. NDS(I)-64 LNNS/ENP 1066
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The specificity of neuronal changes in cerebral infarcts

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.4
Professional: 0.1
Other: 0.3

Project Description:

Objectives: To ascertain the specificity of nerve cell hanges in acutely infarcted brain tissue due to vascular occlusion.

Methods Employed: Temporary and permanent clamping of erebral arteries.

Major Findings: In preliminary studies the acute changes of eurons are evidenced by differences in staining, ranging from allor to dark hue of the cells.

Significance: Since the appearance of neurons forms the asis for an estimation of the resistance of nerve cells to nterrupted blood circulation from heart failure, cerebral ompression, etc., or of the viability of nerve cells after fferent interventions, it is imperative that interpretation of erve cell changes is unequivocal. Among the various morphogic changes, the specificity of an increased stainability with ark color of the cell body is questionable. Likewise, it is oubted that shrinkage of the cell body combined with shrinkage f the nucleus is representative of the anoxic process. The oal is to identify the nerve cell changes which can be regarded as specific to an anoxic condition, so that not only can the exact nature of intracellular alteration be ascertained but also adequate preventive measures can be envisaged.

Proposed Course of Project: Temporary interruption of the cerebral blood flow will be carried out in animals which are known to have separate circulation of blood in each side of the brain. Preliminary results with microembolization and compression of the brain have been reported in a comprehensive review on nonspecific changes in the brain (In: Bourne's *The Structure and Function of Nervous Tissue*, 1972, Vol. 6, pp. 131-251).

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 LNNS/ENP 1449
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The effect of cortisone on the retrograde neuronal changes

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.0
Professional: 0.0
Other: 0.0

Project Description:

Objectives: To determine what effect cortisone treatment may have on the acute reactive phase and the subsequent recovery phase of motor neurons following transection of their axons.

Methods Employed: Cranial motor nerves were cut in different animal species. Serial sections of the brain stem were stained by a combined PAS-gallocyanin method.

Major Findings: Preliminary studies have revealed that the acute peripheral chromatolysis is not influenced by cortisone treatment although the incidence of reactive mitosis is depressed. However, because of subtle differences in the neuronal reaction, new material has been prepared for supplementary studies.

Significance: In view of the frequent use of cortisone, which is known to impair the rate of regeneration of severed peripheral nerves, it is of importance to determine whether the nerve cell body is significantly affected. Until a meticulous survey of the histologic material has been completed no conclusion about the effect of cortisone can be formulated.

Proposed Course of Project: Microscopic analysis of the experimental material which has been prepared.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/ENP 1676
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A comparative study of the area postrema

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.7 |
| Professional: | 0.1 |
| Other: | 0.6 |

Project Description:

Objectives: 1) To determine the effect of chronic meningitis on the topography of the roof of the fourth ventricle, and 2) to determine the effect of chronic trypan blue treatment on the area postrema in cat and monkey.

Methods Employed: Light microscope techniques.

Major Findings: 1) In some animals, such as the guinea pig, the roof of the fourth ventricle has defects while in others, such as the dog, it is free of defects (Z. Anat. Entwicklungsgesch. 131: 861-110, 1970; J. Comp. Neur. 141: 499-512, 1971).

2) The sensitivity of cats and monkeys to apomorphine is depressed by chronic treatment with trypan blue.

Significance: 1) The validity of the statement that the formation of holes in the roof of the fourth ventricle is very marked in man suffering from chronic meningitis with hydrocephalus will be tested by study of the dog, which frequently suffers from severe hydrocephalus (J. Neuropath. Exp. Neur. 20: 386-398, 1961). The formation of holes in the roof of the fourth ventricle as a means to maintain adequate cerebrospinal circulation is still disputed; a study of dog material should clarify current uncertainties about cerebrospinal fluid physiology under pathologic conditions.

2) Since in the normal animal, retching and vomiting are supposed to be induced by apomorphine via chemoreceptors in the intact area postrema, the fortuitous demonstration that cats treated with trypan blue have a lowered sensitivity to apomorphine could be indicative of damage to the area postrema. If this proves to be true, we will indeed have produced an experimental model, without surgical intervention, to test the functional role of the area postrema as a chemoreceptive region not only for the vomiting reaction but also for control of blood pressure. Whether abnormalities in the latter function can be responsible for permanent elevation of the blood pressure, as in hypertensive diseases, would at this stage of experience be conjectural.

Proposed Course of Project: Histologic preparation of brains from dogs suffering from hydrocephalus and from cats chronically treated with trypan blue.

Honors and Awards: None

Publications:

Cammermeyer, J.: Phycomycetes and mast cells in hypependymal cysts of the area postrema in *Macaca arctoïdes*. *Acta Neuropath.* 23: 1-8, 1973.

Serial No. NDS(I)-69 LNNS/ENP 1677
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Comparative anatomical study of mast cells in the area postrema

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.9
Professional: 0.3
Other: 0.6

Project Description:

Objectives: To determine the significance of mast cells in the area postrema.

Methods Employed: Histologic methods for identification of mast cells.

Major Findings: The area postrema contains a large number of mast cells in most of the primates and cats and in many of the rodents. The number varies with age and between species. The mast cells are scattered through the tissue as evidence of a migratory faculty.

Significance: The present observations on the occurrence of mast cells in the area postrema indicate that this cell must be regarded as an inherent but not necessarily an indispensable element in this region; nevertheless, the role of mast cells remains enigmatic. The present results permit selection of species which, because of the rich content of mast cells in the area postrema, are appropriate for future experimental studies.

Proposed Course of Project: Completed.

Honors and Awards: None

Publications:

Cammermeyer, J.: Mast cells in the mammalian area postrema. Z. Anat. Entwicklungsgesch. 139: 71-92, 1972.

Cammermeyer, J.: Migration of mast cells through the area postrema. J. Hirnforsch., in press.

Serial No. NDS(I)-73 LNNS/ENP 2002
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mast cells in the brain

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 1.0 |
| Professional: | 0.2 |
| Other: | 0.8 |

Project Description:

Objectives: To determine the role of mast cells in the central nervous system.

Methods Employed: Examination of microscopic sections from the brains of various animals with the aid of a specific staining technique for mast cells.

Major Findings: Mast cells occur in some species in practically all parts of the brain but with considerable differences between species, regions, the two sides of the brain and different age groups.

Significance: From a preliminary review of material comprising more than 20 different animal species, it appears that the occurrence of mast cells is regulated by a number of factors, the nature of which is still unknown. In general, it can be stated that their presence reflects functional requirements. These cells contain a variety of substances - heparin, histamine, serotonin, dopamine, etc. - which are known to act on coagulability and viscosity of blood, diameter and permeability of blood vessels and neuronal function. The migration of these cells through the tissue permits transportation of biologically active substances to cover a local need. and in this sense the mast cells can be regarded as ancillary transport cells to be called upon for the support of other cellular systems storing various biogenic amines. According to the distribution of mast cells, it is assumed that one of their most significant functions is to assure blood circulation along the surface of the brain and the walls of cerebral ventricles and choroid plexus. The current experience provides a basis not only for selection of appropriate animal species but also for critical evaluation of results.

Proposed Course of Project: The involvement of these cells in the normal cerebral blood flow as well as their possible pathogenetic role in development of local circulatory disturbances in the brain, such as acute and chronic hypertensive encephalopathies, will be explored in experimental investigations.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/ENP 2003
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The thalamo-choroidal body

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.7
Professional: 0.2
Other: 0.5

Project Description:

Objectives: To assess the histologic structure of a region found to be prominent in the cat, and labeled thalamo-choroidal body.

Methods Employed: Various histologic techniques have been used to ascertain the type of constituting elements.

Major Findings: In the cat, a small region situated on either side of the roof over the third cerebral ventricle was found to have a remarkable structure. rich in blood vessels and collagenous fibers, nerve cells, supporting glial elements, plasma cells and mast cells. With increasing age, the number of mast cells decreased and a deposition of amyloid took place.

A similar but diminutive region was noticed in some other animal species also.

Significance: The incidental observation of a special organ at sites of attachment of the roof covering the third ventricle is of interest because of its position near the choroid plexus, which is involved in cerebrospinal fluid circulation. It is speculated that such specialized structures as the thalamo-choroidal bodies serve a chemoreceptive or baroreceptive function for control of blood flow to the choroid plexus. The prominence of this organ in the cat suggests that this species is in possession of a special control mechanism for the blood circulation of the choroid plexus. In this context, it should be noted that a small regulatory organ similar to that described by the principal investigator in the roof of the fourth ventricle in the guinea pig (Z. Anat. Entwicklungsgesch. 131: 86-110, 1970) has been detected by other investigators over the roof of the third ventricle in the rabbit (Leonhardt, Z. Zellforsch. 131: 463-480, 1972). An increasing amount of evidence goes to demonstrate that intracerebral blood circulation is not passive but is regulated by a number of control devices. The manner in which control is performed must be elucidated by supplementary experiments.

Proposed Course of Project: To elucidate the submicroscopic and histochemical composition of the thalamo-choroidal body with other techniques, such as the electron microscope.

Honors and Awards: None

Publications:

Cammermeyer, J.: Organon thalamo-chorioidalis in the taenia thalami of the cat and other mammals. Acta Anat., in press.

Serial No. NDS(I)-73 LNNS/ENP 2004
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The pathogenesis of myelopathies

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.0 |
| Professional: | 0.0 |
| Other: | 0.0 |

Project Description:

Objectives: To assess the various manifestations of spinal cord edema as a factor in the pathogenesis of spinal cord lesions.

Methods Employed: For an orientation of the characteristics of edema within the spinal cord, the volume of each segment of the spinal cord is measured prior to and after immersion in water for varying lengths of time.

Major Findings: 1) When the spinal cord is immersed in fluids, the degree of swelling varies with tonicity of the solute as well s with region.

2) Splitting of the surrounding membranes demonstrated a far reater swelling which in the intact spinal cord is restricted y the membranes.

Significance: Although much attention has centered on the ffect of cerebral edema largely through the many studies of Dr. gor Klatzo, very little is known about the manifestations of pinal cord edema. In some preliminary reviews, the principal nvestigator reported that 1) unlike the brain, the human spinal ord becomes firmer during swelling because of the restrictive action of the superficial membranes (Mschr. Psychiat. Neur. 126: 229-239, 1953), and 2) the cat spinal cord reacts in a similar manner (In: Bourne's The Structure and Function of Nervous Tissue, 1972, Vol. 6, pp. 131-251).

Since resistance to swelling by the unyielding membranes results in abnormal blood circulation, edema can cause spinal cord lesions. Knowledge of the role of edema in the pathogenesis of spinal cord diseases is important for the establishment of guidelines for treatment and prevention of spinal cord edema occurring as a complication to a number of human diseases, such as trauma, viral infection (poliomyelitis), allergic manifestations (multiple sclerosis), cancer, etc. The results of post-mortem experiments purporting to mimic intravital processes will serve as a foundation for additional experimental investigations.

Proposed Course of Project: To develop techniques to determine how blood flow is compromised.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/ENP 2005
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Heredopathia atactica polyneuritiformis (Refsum's disease)

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer, M. D.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0.3
Professional: 0.1
Other: 0.2

Project Description:

Objectives: To summarize the pathologic changes in heredopathia atactica polyneuritiformis (Refsum's disease).

Methods Employed: Routine histopathologic techniques.

Major Findings: 1) Accumulation of fat in brain, liver and kidney; 2) atrophy with formation of "onion bulbs" in peripheral nerves and loss of motor nerve cells in spinal cord; 3) loss of myelin sheaths in certain fiber tracts in the medulla oblongata and the dorsal column of the spinal cord; and 4) loss of nerve cells in the inferior olivary nucleus.

Significance: In the first descriptions of the changes in the rain and the peripheral nerves, the principal investigator (Nord. Med. 29: 617, 1946; addendum in Refsum, Acta Psychiat. eur., Suppl. 38: 1-303, 1946; J. Neuropath. Exp. Neur. 15: 40-361, 1956) predicted that the fundamental lesion was a defect n lipid metabolism, a suggestion which was confirmed in nvestigations by German biochemists, Klenk and Kahlke (Hoppe elyers Z. Physiol. Chem. 333: 133-139, 1963) many years later.

The purpose of this review is to summarize the pathologic changes discerned in the few cases of this rare disease which have come to necropsy and thereby ascertain the nature of characteristic changes.

Although the condition under study is extremely rare, it is the only one in which marked damage of the myelin surrounding the nerve fibers is associated with a definite biochemical defect of genetic origin. However, since after the first description similar peripheral nerve lesions have been described as a complication to other maladies, such as diabetes, the peripheral nerve changes can not be regarded as specific. Efforts to pinpoint the exact nature of the biochemical defect have been carried out simultaneously and independently by investigators in Germany, Switzerland, Norway and the United States without definite results. Thus far, attempts to reproduce the complex of neurologic changes experimentally have been unsuccessful even by prolonged loading of the organism with the lipid in question - phytanic acid (3, 7, 11, 15-tetramethyl-hexadecanoic acid).

Proposed Course of Project: The pathologic changes are being reviewed for inclusion in Handbook of Clinical Neurology at the invitation of the Editors, and the local effect of phytanic acid will be explored in experiments.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-65 LNNS/FN 1231
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Organization of synaptic fields in the mammalian brain

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese, M.D.

Other Investigators: Edward White, Ph.D.
Dennis Landis, M.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMDD

Man Years:

| | |
|---|---|
| Total: | 2.2 |
| Professional: | 1.8 |
| Other: | 0.4 |

Project Description:

Objectives: To map anatomical and functional relationships between synapses in the vertebrate central nervous system. Specific regions of the brain are selected for detailed analysis.

Methods Employed: Appropriately prepared brains are examined by electron microscopy. Three dimensional reconstructions based on electron microscopical examination of serial sections are correlated with the results from examining Golgi impregnated brains with the light and electron microscopes. Alternatively, known sources of synaptic input are destroyed and the degenerated synapses mapped at appropriate times after making the lesion. In the last year, two new techniques have been added to our approach. Leucine $H_3$ autoradiography has been used to identify the origins of synaptic terminals. The acquisition of a freeze-fracture apparatus has made possible examination of the membrane faces of synapses, an aspect not seen with conventional electron microscope sections.

Major Findings: The major new findings this year concern the genetic control of the development of certain synaptic patterns and differentiation of the postsynaptic membrane of excitatory and inhibitory synapses.

In Balb C mice a certain type of cortical synapse was found to be lacking even though the other three types of synapse in this region of the olfactory cortex were present in normal numbers. Furthermore, the normal processes which would have produced the synapse were present so that the Balb C apparently lacks the genetic information to form or maintain a particular synapse. This would be the first demonstration that particular synaptic systems are under genetic control.

The postsynaptic membranes of a variety of known excitatory and inhibitory synapses from olfactory bulb and cerebellum were examined in freeze-fracture faces. Excitatory synapses were found to have an array of particles embedded in the postsynaptic membrane coextensive with the synaptic junction. Similar particles were lacking or not specifically clustered at the synaptic junction of inhibitory synapses.

Significance to Biomedical Research and the Program of the Institute: Much effort in neurological research has been directed to mapping the axonal tracts connecting different areas of cerebral grey matter. Our effort is directed toward understanding what occurs at the termination of these tracts. We have shown previously that complex synaptic patterns exist in various regions of the brain each subserving specific types of physiological activity. This year we have expanded our consideration to the genetic control over the development and maintenance of these synaptic patterns. The knowledge that genetic diseases could be expressed by specific changes in the organization of these systems is helpful in understanding the losses produced by these diseases. Also, knowledge about the types of synaptic interaction in different regions of the central neurons is helpful in understanding the sites of action of various neuropharmacological agents on the mammalian brain. It is particularly relevant in understanding the mechanisms of action of drugs on the nervous system to know the deployment of the various receptors for these drugs. There is reason to believe that our findings of different arrangements of postsynaptic membrane particles in different types of synapse is pertinent to this problem though more direct evidence about the nature of these membrane particles is needed.

Proposed Course of the Project: Dr. Perkins has left and Dr. White will be leaving this year. Definitive publications from both scientists on their work here are in press. This project will be continued with Dr. Landis to complete our analysis of membrane faces of excitatory and inhibitory synapses using the freeze-fracture technique.

Honors and Awards: None

Publications:

Perkins, R.: Synaptic organization of the medical accessory olive. J. Comp. Neurol. 148: 387-415, 1973.

White, E. W.: Synaptic organization of the mammalian olfactory glomerulus: New findings including an intraspecific variation. *Brain Res.* (In Press).

Serial No. NDS(I)-67 LNNS/FN 1442
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Permeability of cellular layers in the vertebrate nervous system

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese, M.D.

Other Investigators: Milton W. Brightman, Ph.D.
Sachio Nabeshima, M.D.
Alan Shabo, M.D.

Cooperating Units: Bureau of Biologics, FDA

Man Years:

| | |
|---|---|
| Total: | 1.3 |
| Professional: | 1.2 |
| Other: | 0.1 |

Project Description:

Objectives: Determination of the pathways taken by colloids and large molecular weight solutes to cross the various cellular layers associated with the nervous system. Currently being studied are: (1) ependyma covering the median eminence and area postrema; (2) arachnoid-dural membrane; (3) arachnoid villae; (4) canal of Schlemm in the primate eye.

Methods Employed: The cytochemical method of Karnovsky is used to localize purified horseradish peroxidase (MW 40,000) after it is injected into various brain or blood compartments of living, anesthetized mammals. The intercellular junctions fronting these compartments are studied with the electron microscope by the colloidal lanthanum or uranyl acetate methods. A new peroxidase of low molecular weight (microperoxidase MW k800) is now being used.

Major Findings: Peroxidase injected into the cerebrospinal fluid of the subarachnoid space in the mouse is able to pass between cells of the pia-arachnoid layer, cross the basement lamina of the brain, and pass between the astrocytic processes forming the limiting surface of the brain to reach the generally open interstitial spaces of the brain. However, peroxidase does not cross from ventricular cerebrospinal fluid into the median eminence and area postrema. The basis of this selective barrier is a system of "tight" junctions within the specialized epithelial cells lining the ventricular surface over these regions. The use of a smaller peroxidase has allowed it to be visualized crossing the capillary pores in the median eminence and area postrema.

A specialized layer of cells joined by tight junctions at the outer border of the arachnoid also prevents peroxidase from reaching the subdural space. This year we have given most of our attention to the arachnoid villae where this arachnoid barrier layer becomes discontinuous at its apposition with the endothelium of the superior sagital sinus. Study of sinus endothelium utilizing tilt microscopy and serial sections has revealed a system of minute pores which are responsible for bulb drainage of cerebrospinal fluid. We are now attempting to determine whether these pores might also be the basis for the valvular action of the arachnoid villae.

Another new ramification of our studies is to examine the meningeal barriers after acute infection with E coli; in these cases the tight junctions between barrier cells open and migration of leuococytes from the dura to the subarachnoid space occurs.

The site of drainage of the aqueous humor from the primate eye has also been examined and evidence produced that the endothelial vacuoles do not provide this channel as previously supposed, but instead it is provided by the endothelial intercellular clefts. The vacuoles have been demonstrated to be an artifact of fixation.

Significance to Biomedical Research and the Program of the Institute: We are determining the permeability to a small protein of the various cellular layers associated with the mammalian nervous system. Our determinations are by means of cytological techniques that show specifically which components of which layers are permeable. Thus we are able to determine the cells as well as the probable mechanisms that control the chemical environment of the brain (blood-brain barrier and blood-cerebrospinal fluid barriers, blood aqueous barriers) in a normally functioning brain and eye. The new findings this year on the arachnoid villae give us specific knowledge about the mechanism of and control over drainage of cerebrospinal fluid. We are beginning to use our knowledge of these normal mechanisms as a basis for studying the changes which occur in pathological conditions such as acute infections of the meninges. Demonstration that the intercellular junctions are the primary site of drainage of aqueous humor from the eye focus attention on these structures rather than the endothelial vacuoles as the probable site of disorder in glaucoma.

Proposed Course of the Project: The work on the arachnoid has been presented to the American Association of Anatomists and the American Association of Neuropathologists. Much of this work is being prepared for publication; some will be incorporated into a Physiological Review which two of the authors are preparing. Dr. Nabeshima has left NIH and reports on his work are being prepared. A paper with Dr. Shabo on the drainage of aqueous humor has been submitted to the American Journal of Ophthalmology. Future work will concentrate on the normal and diseased arachnoid membrane and villae, but this part of the project has been held in abeyance pending arrival of a tilt electron microscope to permit precise study of the numbers and tightness of the intercellular junctions.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/FN 1684
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Structure and function of close intercellular (gap) junctions

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese, M.D.

Other Investigators: Milton W. Brightman, Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.1 |
| Professional: | 0.1 |
| Other: | 0.0 |

Project Description:

Objectives: To study the structure and distribution of gap junctions and to show directly with the electron microscope their permeability to inter and intracellular tracers.

Methods Employed: Peroxidases of various molecular weights are applied to or injected into cells joined by gap or tight junctions and the peroxidase is subsequently localized with the electron microscope. We have elected to work on the crayfish because it has giant nerve cells joined by gap junctions which permit this experiment to be done with simple injection techniques and without damaging the nerve cells.

Major Findings: During this year we concentrated our efforts on two peroxidases, MW 1200 and MW 42,000, which represent the extreme limits in the range of sizes available to us. We injected these peroxidases after, as well as before, fixation and found that the gap junctions are impermeable to both tracers prior to fixation, but permeable after fixation. Thus the size of the cell-to-cell bridges associated with these gap junctions is considerably smaller in life than was expected. This finding, that the size of the intercellular channels in gap junctions increases during fixation, was confirmed this year using fluorescene labeled albumin.

Significance to Biomedical Research and the Program of the Institute: Intercellular electrical coupling has recently been shown to be important at a variety of normal cellular appositions. Changes in this type of coupling appear to be a component of cancerous changes. Our study is an attemt to provide a morphological basis for electrical intercellular coupling by: 1. defining whether this communication occurs directly or via the intercellular spaces; 2. defining what size materials can pass from one cell to adjacent cells; 3. demonstrating directly the cell-to-cell channels that have been thought to be present within gap junctions. Our work over the last year has succeeded in putting an upper limit on the molecular weight of substances that can cross from one cell to another via the gap junctions we have studied, and has showed that these channels either undergo rearrangement of their structure or are supplemented by other channels during fixation for electron microscopy.

Proposed Course of Project: Preliminary results were presented at the annual meeting of the American Association of Anatomists in April, 1971 and will be presented at the Federation Meetings. New findings were presented at the 1972 meeting of the Society for General Physiology and a report is in preparation.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/FN 1881
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Structural basis of synaptic transmission

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese, M.D.

Other Investigators: John Heuser, M.D.
Avery Nelson, Ph.D.
Maryana Henkart, Ph.D.

Cooperating Units: None

Man Years:

Total: 3.1
Professional: 2.6
Other: 0.5

Project Description:

Objectives: To determine the structural basis of transmitter release from synapses.

Methods Employed: (1) Frog sartorius nerve-muscle preparations are exposed to altered ionic environments or stimulated with various programs, and their responses are monitored by standard electrophysiological means until they reach a desired functional state. Then they are immediately fixed and prepared for examination with the electron microscope. Similar experiments are performed in solutions containing horseradish peroxidase and the horseradish peroxidase subsequently localized with the electron microscope. (2) Similar experiments were performed on preganglionic autonomic synapses in the frog. Crayfish stretch receptor synapses were examined in an attempt to find a favorable preparation to study inhibitory synapses.

Major Findings: The traditional fine-structural appearance of the synapse represents a resting or equilibrium state. With intense electrical stimulation and transmitter release approaching exhaustion, the intracellular organelles of the presynaptic terminal undergo well-defined, reversible changes. These include mitochondrial swelling and migration to new regions, depletion of synaptic vesicles with coincident enlargement of the presynaptic membrane surface, and finally formation of coated vesicles and Golgi-apparatus-like bodies, apparently as intermediate states in the recycling of membrane back into synaptic vesicles.

When similar experiments are done in solutions containing horseradish peroxidase, the tracer progressively fills various membrane forms inside the synapse, thus tracing directly the recycling of membrane from coated vesicles to synaptic vesicles and back to surface membrane concomitant with transmitter release.

Identical results were obtained with autonomic synapses from the sympathetic ganglion indicating that new synaptic vesicles here are also formed by recycling of membranes. Examination of the inhibitory synapses on crayfish stretch receptor cells revealed that these synapses receive synaptic input from other sources. An incidental result of work on neuromuscular synapses in the rat is that RNA synthesis which accompanies overload hypertrophy is largely the result of proliferation of connective tissue cells.

Significance to Biomedical Research and the Program of the Institute: This work contributes to an understanding of the role of membranes in the storage and release of transmitter at synapses and shows that the mechanism for release of neurotransmitters is similar to that employed for release of glandular secretions. Also this work helps to broaden the general anatomical criteria for identifying synapses, thereby aiding future attempts to define the existence and location of functional contacts in the CNS, and most important, permitting attempts to define their physiological state. The discovery that the RNA synthesis in muscle hypertrophy is the result of proliferation of connective tissue cells casts doubt on the idea that muscle cells proliferate in hypertrophy.

Proposed Course of Project: The work on transmitter release at the neuromuscular synapse has been completed and presented at several scientific meetings. It has also appeared as a full length paper. The work on the sympathetic ganglion is being presented at meetings this spring and will be completed within the next year. The work on the crayfish inhibitory synapse has been submitted to the Proceedings of the National Academy of Science. The work on muscle hypertrophy has appeared as a full length paper. Drs. Heuser and Henkart have left NIH and their aspects of the work terminated. The future course of this project will be to complete the work on the autonomic synapses.

Honors and Awards: None

Publications: Heuser, J. E., and Reese, T. S.: Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. J. Cell Biol. (In Press)

Jablecki, C. K., Heuser, J. E., and Kaufman, S.: Autoradiographic localization of new RNA synthesis in hypertrophying skeletal muscle. J. Cell Biol. (In Press)

Serial No. NDS(I)-71 LNNS/NC 1879
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Chemical changes in brain of transplacentally methylazoxymethanol (MAM) induced microencephaly in rats

Previous Serial Number: Same

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Gert L. Laqueur, M.D.
Grazyna Szumanska, Ph.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMD

Man Years:

| | |
|---|---|
| Total: | 1.7 |
| Professional: | 1.0 |
| Other: | 0.7 |

Project Description:

Objectives: In previous studies of microencephaly, a quantitative and qualitative disturbance of myelin formation was found in cerebral cortex, hippocampus, diencephalon, metencephalon, and medulla oblongata. Furthermore, a diminished amount of total lipids and phospholipids was observed in these brains, as compared to controls. The present investigation was concerned with the fractionation of the phospholipids and glycolipids as a continuation study of lipid composition change observed in microencephalic brains during maturation and adulthood.

Methods Employed: The microencephaly was induced by simple intraperitoneal injection of 20 mg/kg of MAMA on the 15th day of gestation. The control animals were treated similarly but received saline instead of MAMA. The experimental and control rats were sacrificed at 28, 60, and 90 days of age. Lipid extraction was carried out with chloroform-methanol 2:1. A combination of DEAE column and thin layer chromotography was used for the determination of phospholipid fractions, cerebrosides, and sulfatides.

Major Findings: In microencephaly, a low concentration of phosphatidyl ethanolamine on wet and dry basis in the brain was found when this value was compared with the one obtained from control rats. However, no changes were seen in the cerebral content of phosphatidyl choline, phosphatidyl serine, and sphyngomyelin in these animals. Therefore, the reduction of phosphatidyl ethanolamine was most likely responsible for the observed decrease of the total phospholipids in the microencephalic rats. In addition, the cerebral sulfatide and cerebroside concentrations were the same in the microencephalic as in control animals.

Significance to Biomedical Research and the Program of the Institute: Upon completion of these studies, one might be able to determine whether MAM has a primary and/or secondary effect on myelin formation. In any case, these investigations might be helpful in the understanding of some neurological conditions in which (1) abnormal synthesis or (2) degeneration of myelin takes place. Therefore, this or a similar model system will be useful for comparative studies of abnormal myelin formation observed in (1) malnutrition and (2) help in elucidating demyelinizing processes which may play a role, for example, in multiple sclerosis.

Proposed Course of the Project: Future investigations of MAM microtoxicity should be concerned with evaluating the possible direct effect of MAM on (1) myelin synthesis; (a) in vivo, (b) in vitro, and (2) testing of MAM effect on chromosomes in fetal brain in vitro.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/NC 1880
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Glucose brain uptake following osmotic blood-brain barrier (BBB) injury

Previous Serial Number: Same and incorporating Serial No. NDS(I)-72 LNNS/CP 1964

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Igor Klatzo, M.D.
Zbigniew M. Rap, M.D.
Stanley I. Rapoport, M.D.

Cooperating Units: Laboratory of Neurophysiology, NIMH

Man Years:

| | |
|---|---|
| Total: | 1.7 |
| Professional: | 1.0 |
| Other: | 0.7 |

Project Description:

Objectives: Intracarotid hypertonic urea perfusion produced two groups of blood-brain barrier (BBB) damage. In the first group, the BBB damage was irreversible, associated with brain edema, hyperemia, and an increased uptake of 2-deoxy-D-glucose by passive diffusion. In the second group, the BBB damage was reversible or partially reversible and the observed stimulation of 2-deoxy-D-glucose uptake occurred by facilitated diffusion.

This continuous investigation was undertaken in order (1) to clarify whether the increased facilitated diffusion of 2-deoxy-D-glucose was specific for urea or it was the result of hypertonic treatment, (2) to assess better the function of BBB glucose transport following hypertonic perfusion by eliminating the possible metabolic effect of 2-deoxy-D-glucose and using the nonmetabolizing hexose.

Methods Employed: Young adult rabbits were used for these experiments. Intracarotid cannula was inserted in one of the common carotid arteries, the external carotid was ligated in order to perfuse directly into the internal carotid artery. Double isotope technic was applied by injecting a mixture of $^3H_2O$ and $^{14}C$ 2-deoxy-D-glucose or 3-0 methyglucose ten minutes after damaging the BBB with intracarotid hypertonic urea, acetamide and NaCl perfusion. For the inhibition studies cold 2-deoxy-D-glucose or phlorizin was injected simultaneously with the labeled 2-deoxy-D-glucose or 3-0 methylglucose respectively. The rabbits were sacrificed by decapitation 15 seconds later. The brain samples were taken from each temporo-lateral region, homogenized, weighed, and solubilized for radioisotope counting in liquid scintillation counter.

The brain uptake index was calculated per 100 mg of brain tissue.

$$\text{BUI} = \frac{\text{Tissue } ^{14}\text{C} \;/\; \text{Tissue } ^{3}\text{H}}{\text{Injected Mixture } ^{14}\text{C} \;/\; \text{Injected Mixture } ^{3}\text{H}} \times 100$$

Major Findings: The increased brain uptake of 2-deoxy-D-glucose was observed after intracarotid perfusions of hypertonic acetamide and NaCl as well as after urea. The increased entry of 2-deoxy-D-glucose was self-inhibited when cold 2-deoxy-D-glucose was injected simultaneously with the labeled compound indicating an increase in facilitated diffusion of 2-deoxy-D-glucose from blood to brain. Similar findings were observed when the possible metabolic effect of 2-deoxy-D-glucose was eliminated by using the unmetabolizable 3-0 methylglucose. Furthermore, the increased uptake of the labeled 3-0 methylglucose was inhibited by phlorizin (the known inhibitor of glucose transport carrier) when the $^{14}$C labeled 3-0 methylglucose was simultaneously injected with cold phlorizin in progressively greater concentrations. These findings strongly indicate that the stimulation of facilitated glucose transport from blood to brain is the result of the hyperosmotic rather than specific chemical treatment. However, the mechanism responsible for the increase in the facilitated diffusion is still unknown and deserves further investigation.

Significance to Biomedical Research and the Program of the Institute: These findings are of great interest from both the physiological and pathophysiological point of view of the BBB. (A) This is the first demonstration of stimulation in facilitated diffusion of glucose from blood to brain although such a hypertonic stimulation of glucose transport was described in tissue, muscle, and yeast cells. (B) Since glucose is one of the most important nutrients of the brain, the widening of the knowledge concerning its passage from blood to brain in health and disease is of major importance. (C) It may help in elucidating the pathogenesis of many neurological disorders. (D) It may offer an understanding and a possibility to select the best therapeutic approach to a given disease.

Proposed Course of Project: The evaluation of the BBB function following hyperosmotic injury is almost completed as far as the effect on glucose transport is concerned. This model may be useful in the investigation of hypertonic effects on amino acids and biogenic amines transport from blood to brain. The project "Sequelae of Osmotic Blood-Brain Barrier (BBB) Damage" (Serial No. NDS(I)-72 LNNS/CP 1964) was incorporated with this project.

Honors and Awards: None

Publications:

Spatz, M., Rap, Z. M., Rapoport, S. I., and Klatzo, I.: The effects of hypertonic urea on the blood-brain barrier and on the glucose transport in the brain. In Reulen, K. J. and Schürmann, H. (Eds.): Steroids on Brain Edema. Berlin, Germany, Springer-Verlag, 1972, pp. 19-27.

Serial No. NDS(I)-73 LNNS/NC 1999
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Brain uptake of radiolabeled 2-deoxy-D-glucose in ischemic gerbils

Previous Serial Number: None

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Gwan K. Go, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.55 |
| Professional: | 0.55 |
| Other: | 1.0 |

Project Description:

Objectives: The Mongolian gerbils possess a high susceptibility for the development of cerebral infarction following unilateral ligation of the common carotid artery in contrast to other tested animals. This study was undertaken to evaluate the effect of ischemia on glucose transport from blood to brain as one of the multidisciplinary approaches for the pathophysiological investigation of cerebrovascular disorders.

Methods Employed: Several groups of gerbils were subjected to left common carotid artery clipping for various periods of time and the release of clip for five minutes. Thereafter, a double isotope technique was used for measuring the differential uptake of tracers injected as a mixture intracarotidally 15 seconds prior to the decapitation of the animals. In this method $^3H_2O$ was used as a reference with which the uptake of $^{14}C$ label 2-deoxy-D-glucose was compared. For the inhibition studies the injected mixture also contained various concentrations of unlabeled (cold) 2-deoxy-D-glucose. The brain uptake index was calculated as follows:

$$\text{BUI} = \frac{\text{Tissue } ^{14}\text{C} \ / \ \text{Tissue } ^{3}\text{H}}{\text{Injected Mixture } ^{14}\text{C} \ / \ \text{Injected Mixture } ^{3}\text{H}} \quad \text{X } 100$$

Major Findings: An increased brain uptake of 2-deoxy-D-glucose was found in 40, 80, and 100% of both sensitive (with cerebral symptoms) and nonsensitive (without cerebral symptoms) gerbils after left common carotid ligation for 3, 6, and 18 hours respectively. The brain uptake index (BUI) for 2-deoxy-D-glucose was as follows: 51.88 ± 1.96 S.E. (N=6) in controls, 66.02 ± 2.18 S.E. (N=5) at 3 hours, 77.74 ± 7.88 S.E. (N=6) at 6 hours, and 88.2 ± 1.17 S.E. (N=6) at 18 hours after left common carotid clipping. In controls and experimental animals self-inhibition was demonstrated with simultaneous injection of cold 2-deoxy-D-glucose in progressively greater concentrations. A difference in the degree of inhibition was found between the sensitive and nonsensitive gerbils at 18 hours following the occlusion of common carotid artery.

The self-inhibition of the stimulated brain 2-deoxy-D-glucose uptake in the ischemic gerbils suggested that (1) the BBB passage for 2-deoxy-D-glucose is saturable, and (2) the transfer most probably took place by facilitated diffusion except for 18 hours sensitive gerbils in which passive diffusion occurred, too.

Significance to Biomedical Research and the Program of the Institute: The basic comprehension of blood-brain barrier (BBB) function in ischemia is of major importance (1) for the understanding of the pathophysiological process occurring in cerebrovascular disease and many other neurological disorders, and (2) may be helpful in selecting the best therapeutic approach to a given disease.

Proposed Course of the Project: The study of glucose brain uptake in ischemic gerbils is still in progress. Many data have been collected and will have to be evaluated to clarify whether the increase uptake of 2-deoxy-D-glucose is due to transport only or also due to metabolic alterations. This model system will be useful also in studying the effect of ischemia on the transport of amino acids and isogenic amines.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/NC 2000
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cerebral edema and blood-brain barrier impairment to protein tracers in cerebral ischemia in the gerbils

Previous Serial Number: None

Principal Investigator: Gwan K. Go, M.D.

Other Investigators: Maria Spatz, M.D.
Igor Klatzo, M.D.
Hanna Pappius, Ph.D.

Cooperating Units: Montreal Neurological Institute

Man Years:

| | |
|---|---|
| Total: | 1.88 |
| Professional: | 0.88 |
| Other: | 1.00 |

Project Description:

Objectives: In human cerebral ischemia, brain edema is considered to be an important factor in causing mortality (Shaw, Alvord, and Berry, 1959). Experimentally, cerebral ischemia can be easily produced in Mongolian gerbils by ligation of a single common carotid artery (Levine and Payan, 1966 and Kohn, 1972). Therefore, the gerbils were chosen for the study of cerebral edema and disruption of the blood-brain barrier (BBB) in ischemia.

Methods Employed: In both experiments, several groups of adult gerbils were subjected to unilateral clipping of the common carotid artery for various periods of time. Before termination of each experiment, the gerbils were evaluated for the presence of cerebral symptoms. (1) For the edema study, the brain was removed quickly and the separated hemispheres weighed immediately (fresh weight). After drying at 98°C for six days, the samples were weighed again to constant weight (dry weight). Thereafter, the tissues were saved for the determination of cerebral electrolytes. (2) In the study of the blood-brain barrier (BBB) impairment to protein tracers, the gerbils were injected with 20% solution of Evans Blue (2g/Kg body) into the femoral vein prior to the occlusion of the left carotid artery. The removed brains were inspected for the evidence of parenchymotous blue staining prior and after fixation of the brain in 10% buffered formalin.

Major Findings: Symptoms of cerebral infarction were observed in 25-50% of animals with ligation of the left common carotid artery. The left hemisphere of the sensitive animals showed a decreased dry weight percentage (i.e. an increased water content) when compared with those of the controls. The content of water increased with the duration of arterial occlusion. Furthermore, the right hemispheres of the sensitive animals did not differ from those of the control animals.

The infiltration of cerebral parenchyma by Evans Blue stain was seen in various degree in the group of 18 hours of carotid occlusion, only. The late occurrence of BBB disruption in cerebral ischemia in gerbils resembled the human conditions in which, after vascular obstruction, an exudation of radioactive tracers was observed between first and second week (Usher and Quinn, 1969).

Significance to Biomedical Research and the Program of the Institute: Cerebral edema occurs as one of major complications of many neurological disorders such as ischemia, trauma, tumors, chemical poisoning and others. The basic understanding of the type of edema and its development is very crucial for the clinician who is faced with choosing the appropriate treatment. Various investigations of this problem are essential for finding the factor or factors responsible for the occurrence of cerebral edema and its treatment.

Proposed Course of the Project: This study is still in progress. The immediate plans are underway to determine the electrolyte content of brain in cerebral ischemia of gerbils.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/NC 2001
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The effect of $pO_2$, $pCO_2$, and pH on glucose transport in the brain

Previous Serial Number: None

Principal Investigator: Frank Berson, M.D.

Other Investigators: Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.3 |
| Professional: | 1.3 |
| Other: | 0.0 |

Project Description:

Objectives: The first observation of increased facilitated glucose transport in the brain was described in hyperosmotic blood-brain barrier injury of rabbits. A similar stimulation of 2-deoxy-D-glucose was seen in ischemic gerbils. It is the aim of this investigation to evaluate the effect of individual and combined factors such as hypoxia, hypercapnia, pH and blood flow which may play a role in modulation of glucose transport from blood to brain.

Methods Employed: Young adult rabbits under pentothal anesthesia were used for these experiments. Each rabbit underwent tracheotomy and its ventilation was regulated with a small animal respirator. The right carotid artery was exposed and cannulated in preparation for a later injection of a given isotope mixture. The right femoral artery was cannulated for the dual purpose of (1) monitoring arterial blood pressure, and (2) removing arterial blood sample for blood gas analysis. After 15 minutes of ventilation to stabilize the animal at normal levels of oxygenation and $CO_2$ content, the inspired gas was changed to create various experimental level $pO_2$, $pCO_2$, and pH and the ventilation continued for an additional 15 minutes. At the end of this period, the blood pressure was again checked and a second arterial blood sample was drawn for gas analysis. Finally a mixture of $^3H_2O$ with $^{14}C$ labeled 2-deoxy-D-glucose was injected through the intracarotid polyethylene tube 15 seconds prior to the decapitation of the animal. A sample for each side of the brain was processed for the counting of radioactivity in Beckman's liquid scintillation counter. The arterial blood samples were then analyzed for $pO_2$, $pCO_2$, and pH levels in a blood gas analyzer.

Major Findings: The technical setup for the development of such a model with different groups of animals took a relatively long time. The actual experiments for testing the glucose transport under well defined conditions began and data is being collected. So far, the observations which were made indicate that severe hypoxia and/or hypercapnia decrease the brain uptake for 2-deoxy-D-glucose.

Significance to Biomedical Research and the Program of the Institute: The study of BBB function under pathological condition is essential for the understanding of many disease processes in the brain. These investigations are especially focused on determining the function of BBB and the specifically altered respiratory conditions which are commonly associated with cerebral ischemia, trauma, and also many systemic diseases. It is hoped that the observation made in these and similar studies will not only be helpful in recognizing the crucial factor or factors in a disease process, but also in preventing the deterioration of the patient's condition.

Proposed Course of the Project: The testing of the nonmetabolizing 3-0 methylglucose brain uptake will be included to find out whether the changes are only due to transport or also due to altered metabolism. This model will also be used to study the active transport of amino acids in the brain.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-66 LNNS/NC 1304
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Pinocytosis in the brains of dead rats

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman, Ph.D.

Other Investigators: None

Cooperating Units: None

This project has been canceled.

Serial No. NDS(I)-67 LNNS/NC 1443
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The intracerebral movement of proteins injected into the blood and cerebrospinal fluid of rodents

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman, Ph.D.

Other Investigators: Thomas S. Reese, M.D.
Masaharu Hori, M.D.
Stanley Rapoport, M.D.
Igor Klatzo, M.D.

Cooperating Units: Laboratory of Neurophysiology, NIMH

Man Years

| | |
|---|---|
| Total: | 0.9 |
| Professional: | 0.6 |
| Other: | 0.3 |

Project Description:

Objectives: To ascertain the effects of hypertonic salt solutions on the blood-brain barrier to peroxidase.

Methods Employed: Hypertonic (3.0 M) solutions of urea are applied topically to the cerebral cortex of rabbits or injected into the carotid artery. Trypan blue and horseradish peroxidase (HRP) are injected into the peripheral circulation just before the urea is administered.

Major Findings: The blood-brain barrier to trypan-blue-protein and to HRP is broken by a slight separation of endothelial junctions in cerebral blood vessels.

Significance to Biomedical Research and the Program of the Institute: Since there is no injury to the endothelium and only the junctions are affected reversibly, this procedure may be an efficacious means of bringing therapeutic drugs to nerve tissue that is otherwise isolated from the bloodstream.

Proposed Course of Project: To verify the findings in rabbits where 3.0 M urea is topically applied to the pial vessels and to compare the effects of isotonic urea and hypertonic urea injected into a carotid artery.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 LNNS/NC 1587
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A blood-brain-barrier to peroxidase in the normal and injured brain of elasmobranchs

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman, Ph.D.

Other Investigators: Thomas S. Reese, M.D.
Lise Prescott, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.1 |
| Other: | 0.1 |

Project Description:

Objectives: The results of the first phase have been completed and published, but it now must be made certain that the perivascular glia is the barrier by perfusing peroxidase retrogradely, i.e. from the cerebral ventricle.

Methods Employed: Exogenous horseradish peroxidase (HRP) is to be perfused ventricuilo-cisternally in sharks and dogfish and its cytological distribution ascertained electronmicroscopically.

Major Findings: In two poorly-fixed specimens, HRP reached the perivascular basement membrane from the cerebral ventricles.

Significance to Biomedical Research and the Program of the Institute: If HRP reaches the basement membrane in well-fixed specimens, then the glial junctions are not barriers after all, or are one-way valve-like barriers, or the basement-membrane is the barrier.

Proposed Course of Project: To procure healthy specimens and repeat the ventricular perfusions to determine the differential roles of basement membrane and glial junctions as extracellular barriers to protein passage.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/NC 1678
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Distribution of exogenous proteins in brain tumors

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman, Ph.D.

Other Investigators: L. Prescott, M.D.
T. S. Reese, M.D.
N. Vick, M.D.
D. Bigner, M.D.

Cooperating Units: Medical Neurology Branch, NINDS

Man Years

| | |
|---|---|
| Total: | 1.4 |
| Professional: | 1.1 |
| Other: | 0.3 |

Project Description:

Objectives: To determine differences in permeability of the endothelium in neoplasms to peroxidase and the availability of the extracellular spaces (ecs) of the tumors to this protein.

Methods Employed: Rous sarcoma virus (RSV) or Simian virus 40 ($SV_{40}$) are inoculated into the brains of newborn hamsters and, after the appearance of ataxia, circling or somnolence, peroxidase is injected intravascularly and the fixed tumor examined electronmicroscopically.

Major Findings: The large extracellular spaces of both tumors are quickly entered by peroxidase which crosses the vessels of the $SV_{40}$ tumor via open endothelial junctions and probably via endothelial fenestrae which outnumber the junctions four to one.

Significance to Biomedical Research and the Program of the Institute: The above elucidation of how substances, including chemotherapeutic agents, reach tumor cells is a prerequisite in devising means for their eradication.

Proposed Course of Project: To obtain more $SV_{40}$ specimens as controls for endogenous peroxidatic activity so as to complete this study; to examine the RSV material with a tilt stage in order to resolve whether the endothelial junctions have a narrow but patent gap.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/NC 1805
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A reinvestigation of central nervous system fine structure with freeze-replication techniques

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman, Ph.D.

Other Investigators: Lise Prescott, M.D.
Thomas S. Reese, M.D.
H. Gainer, Ph.D.
J. L. Barker, M.D.

Cooperating Units: Behavioral Biology Branch, NICHD

Man Years:

| | |
|---|---|
| Total: | 0.9 |
| Professional: | 0.6 |
| Other: | 0.3 |

Project Description:

Objectives: To examine intercellular junctions in the choroid plexus under hyperosmotic conditions which may lower the blood-CSF barrier and to see membrane changes associated with exocytosis in neurosecretory systems.

Methods Employed: Freeze-replication of samples from choroid plexus and Aplesia neurosecretory axons have been made.

Major Findings: These projects have been initiated within the past several weeks.

Significance to Biomedical Research and the Program of the Institute: The elucidation of membrane changes during manipulations designed to lower the blood-CSF barrier and during release of neurohormones.

Proposed Course of Project: To proceed with the experiments.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-72 LNNS/NC 1965
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The effect of biogenic amines on blood-brain barrier to peroxidase

Previous Serial Number: None

Principal Investigator: Erik Westergaard, M.D.

Other Investigators: Milton W. Brightman, Ph.D.

Cooperating Units: None

Man Years

| | |
|---|---|
| Total | 1.5 |
| Professional: | 1.2 |
| Other: | 0.3 |

Project Description:

Objectives: To determine the effects of biogenic amines on the permeability of cerebral vessels to exogenous peroxidase after first establishing the degree of permeability in normal vessels.

Methods Employed: Intravascular injection of horseradish peroxidase (HRP) followed by ventriculocisternal perfusion of amines and electron-microscopic evaluation of HRP distribution.

Major Findings: In normal mice, rats, and hamsters given dialyzed peroxidase and ferritin intravenously, certain pial and parenchymal arterioles transfer the proteins in vesicles to the perivascular spaces. Serotonin and norepinephrine appear to increase this transfer.

Significance to Biomedical Research and the Program of the Institute: This unexpected transfer of protein across normal cerebral arterioles implies a route for immune globulins or antigens and might provide a basis for initiation of possible auto-immune reactions in the central nervous system.

Proposed Course of Project: The results are being prepared for publication. Further experiments on the effects of biogenic amines will be done for corroboration of our earlier results.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 LNNS/NR 1054
1. Neuropathology and Neuroanatomical Sciences
2. Section on Nerve Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Trophic functions of the peripheral nervous system

Previous Serial Number: Same

Principal Investigator: Lloyd Guth, M.D.

Other Investigators: R. Wayne Albers, Ph.D., Dou Huey Jean, Ph.D.

Cooperating Units: Laboratory of Neurochemistry, NINDS

Man Years

| | |
|---|---|
| Total: | 1.8 |
| Professional: | 1.0 |
| Other: | 0.8 |

Project Description:

Objectives: To investigate the mechanisms by which the nerve regulates the biochemical composition and metabolism of the muscle fiber.

Methods Employed: Altered function in the rat's soleus was produced by inactivating antagonistic muscles via transection of the common peroneal nerve. These procedures were compared to the effect of cross-reinnervating the slow soleus muscle with the peroneal nerve (which normally supplies the fast muscles). The properties of the soleus muscle myosin were evaluated by determining ATPase activity and peptide composition.

Major Findings: Following peroneal nerve transection, the soleus muscle became faster and exhibited an increased proportion of fibers having high myofibrillar ATPase activity. Biochemically, the myosin showed increased ATPase activity, and tryptic digestion of the myosin produced an electrophoretic pattern of eight peptide bands. Normal fast muscle myosin has these eight peptide bands whereas normal soleus has only seven of them. Consequently, the operative procedure produced a change in the qualitative nature of the myosin molecule. Similar changes occurred after cross-reinnervation of the soleus muscle with the peroneal nerve.

Significance: The cross-reinnervation experiments performed in laboratories throughout the world have all indicated that the histochemical, biochemical, and physiological properties of slow and fast muscles can be interconverted by cross-union of the nerves that normally supply these muscles. From such studies as these, a specific "trophic" function has been ascribed to the neuron. However, in none of these experiments were the critical control procedures performed to rule out effects produced merely by the transection of the nerve. Our finding here is that transection of the peroneal nerve induces a transformation in the myosin of the soleus muscle. This transformation is probably sufficient to account for the observed physiological changes. Since the structure of myosin can be altered by procedures other than nerve cross union, the evidence for a specific neurotrophic regulation of myosin can no longer be considered compelling, and must be reevaluated experimentally.

Proposed Course of Project: To be continued during the coming year.

Honors and Awards: None

Publications:

Guth, L.: Regulation of metabolic and functional properties of muscle. In Goss, R. (ed.): *Regulation of Organ and Tissue Growth*. New York, Academic Press, 1972, pp. 61-75.

Guth, L.: A review of the evidence for the neural regulation of gene expression in muscle. In Podolsky, R. L. (ed.): *Contractility of Muscle Cells and Related Processes*. Englewood Cliffs, N. J., Prentice-Hall, Inc., 1971, pp. 189-201.

Guth, L. and Samaha, F. J.: Erroneous interpretations which may result from application of the "myofibrillar ATPase" histochemical procedure to developing muscle. *Exp. Neurol*. 34: 465-475, 1972.

Guth, L. and Wells, J B.: Physiological and histochemical properties of the soleus muscle after denervation of its antagonists. *Exp. Neurol*. 36: 463-471, 1972.

Jean, D. H., Guth, L., and Albers, R. W.: Neural regulation of the structure of myosin. *Exp. Neurol*. 38: 458-471, 1973.

Serial No. NDS(I)-66 LNNS/NR 1303
1. Neuropathology and Neuro-anatomical Sciences
2. Section on Nerve Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Elucidation of factors influencing the characteristics of muscle sensori - motor components as revealed by histochemical and other analysis

Previous Serial Number: Same

Principal Investigator: Herbert Yellin, Ph.D.

Other Investigators: Danny A. Riley, Ph.D.

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 2.2 |
| Professional: | 2.0 |
| Other: | 0.2 |

Project Description

Objectives: To determine the histochemical changes in skeletal muscle fibers following (a) injury and (b) alterations in such physiological influences as hormones and tension.

Methods: Frozen cross-sections of muscles were stained for myofibrillar ATPase (alkali-pretreatment), intermyofibrillar ATPase (acid-pretreatment) and succinic dehydrogenase. These techniques were applied in the following experiments.

1. Guinea pig muscles were injured by stab wounds or by freezing. The sequence of changes from 1-120 days was analyzed.

2. The normal and denervated levator ani muscles of male rats were examined 0-30 days after castration and after testosterone injection into castrated and normal rats.

3. The phrenic nerve was transected unilaterally in rats. This procedure produces a transient hypertrophy of the denervated hemidiaphragm, which is maximal at 1 week. Muscle fiber types were assessed from 1-4 weeks after surgery.

Major Findings:

1. During the initial degenerative phase the injured portion of fibers fail to stain for ATPase after either alkali or acid pretreatment, whereas the uninjured portions continue to stain normally. With the commencement of fiber restoration the injured regions acquire prominent staining for ATPase after either alkali or acid pretreatment, and with a myofibrillar distribution characteristic of developing fibers. Thereafter, the once injured region gradually assumes the adult staining pattern. The same changes are observed in injured regions of denervated muscles.

2. Hormone augmentation causes the levator ani muscle to undergo appreciable hypertrophy when the muscle is innervated, and appreciable recovery of mass subsequent to denervation.

3. The denervated hemidiaphragm, subjected to periodic stretch by contractions of the contralateral intact hemidiaphragm, undergoes transient hypertrophy (peak - 7 days) followed by regression and atrophy. The several fiber-types of the diaphragm exhibit different rates and degrees of hypertrophy and involution. The tonic, red muscle fiber hypertrophies to the greatest degree and for the longest duration. The phasic, pale fiber exhibits the least hypertrophy and for the shortest duration.

Significance:

1. The histochemical characteristics of injured skeletal muscle fibers suggest restorative processes by myogenic elements that are similar to embryonic myoblasts. These cells may fuze with or grow from non-injured portions of the muscle fiber. The uninjured regions retain their original differentiated characteristics while the injured regions go through a developmental sequence quite comparable to that seen in ontogeny. This shows that adult muscle is capable of considerable plasticity--it presumably can alter its appearance by transformation of existing fibers and by formation of new fibers. The extent to which each of these processes occurs during remodelling of muscle is a most important subject for future studies.

2. The innervation is generally held to be of primary importance in sustaining skeletal muscle. However, the profound anabolic effect of testosterone on the innervated as well as denervated levator ani muscle (a hormone-sensitive muscle) illustrates the diverse factors which may be responsible for the fundamental well being of a variety of muscles.

3. We know that the red, tonic muscle fibers are less affected than the pale, phasic fibers by denervation, but that the former are more adversely affected by tenotomy. The observed

transient hypertrophy and slow regression of the red, tonic fibers in the periodically-stretched denervated hemidiaphragm further illustrates the differential sustaining influence of tension on the several types of effector units within a muscle.

Proposed Course of Project: Study at the subcellular level the sustaining influence of innervation, tension, and humoral substances upon muscle.

Honors and Awards: None

Publications:

Yellin, H.: Differences in histochemical attributes between diaphragm and hindleg muscles of the rat. Anat. Rec. 173: 333-340, 1973.

Hall-Craggs, E. C. B., Guth, L., and Yellin, H.: The "tonico-phasic" muscle: an experimental model. Exp. Neurol. 35: 398-401, 1973.

Riley, D. A. and Allin, E. F.: The effects of inactivity, programmed stimulation, and denervation on the histochemistry of skeletal muscle fiber types. Exp. Neurol. (In Press) 1973.

Serial No. NDS(I)-68 LNNS/NR 1586
1. Neuropathology and Neuroanatomical Sciences
2. Section on Nerve Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Trophic nerve function of transplanted neurons

Previous Serial Number: Same

Principal Investigator: Andrew A. Zalewski, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 1.0 |
| Others: | 1.0 |

Project Description:

Objective: The results of previous studies demonstrated that neurons survived in autografts of peripheral ganglia placed into the anterior chamber of the eye or into the sternomastoid muscle. Importantly, neurons also survived in homografts provided that donor and host exhibited major histocompatibility. The present studies were performed to determine whether transplanted neurons were functional, that is could these neurons regenerate their nerve fibers and establish a functional reinnervation.

Methods Employed: The functional evaluation of transplanted neurons was ascertained by determining whether they could induce the formation of taste buds. Specifically, the sensory vagal nodose of the rat was placed into the anterior chamber of the eye and a graft of the tongue's vallate papilla added to it. The only way taste buds could appear in the tongue graft would be for the transplanted neurons to regenerate their nerve fibers into it and transform the squamous epithelial cells into taste bud cells.

In a related study the nodose ganglia was implanted into the sternomastoid muscle and joined to the cut distal end of the host's nerve which innervates the epiglottis. This study was designed to determine whether transplanted neurons could regenerate their nerve fibers over great distances and still retain their ability to induce taste bud formation.

Finally, different types of sensory (e.g., lumbar) and sympathetic (e.g.. superior cervical) ganglia were combined with different types of tongue (e.g.. dorsal or ventral surface) for skin grafts to determine any neuron or tissue specificities involved in taste bud formation.

Major Findings:

1. Neurons in autografts were able to regenerate their nerve fibers into the vallate papillae grafts and cause taste bud formation.

2. Neurons in homografts also caused bud regeneration despite the presence of an immune reaction.

3. Homografted neurons can utilize the nerve trunks of the host to regenerate long distances while retaining the ability to form a functional connection. This was shown by the fact that neurons from homografted ganglia placed into muscle grew fibers through the host's nerve to the epiglottis and caused bud regeneration.

4. Neurons in transplanted lumbar ganglia were able to induce bud regeneration but those in transplanted sympathetic ganglia were not.

5. Regenerated taste buds were found in tongue grafts taken from the dorsal or ventral surface of the tongue. No buds formed in skin grafts despite reinnervation by neurons which caused bud regeneration in tongue grafts.

Significance:

1. The above findings demonstrate that neurons which survive transplantation are functional in that they can regenerate their nerve fibers and establish a functional innervation.

2. Homografted neurons can survive and function despite the presence of an immune reaction.

3. Homografted neurons can regenerate nerve fibers over great distances by growing through nerve trunks of the host. Apparently no adverse conditions exist whereby neurilemmal, fibroblasts or other cells of the host's nerve trunk inhibit nerve regeneration of the homografted neurons. Similarly, no histoincompatibility factors discourage the relationship required between homografted neuron and end-organ to form a functional connection since nerve fibers of the homografted neurons grew into host's tongue epithelium and caused taste bud formation. This means that the major factor influencing the success of neuronal

homografting is the immune reaction and not local graft-host tissue interactions. It thus seems possible that in time neuronal grafts could be used to restore function that is lost by diseases which cause neuron death.

4. Since neurons in lumbar ganglia grafts caused bud regeneration it seems that all sensory ganglia have gustatory (i.e., taste bud inducing) neurons. Heretofore, only specific cranial sensory ganglia were credited with gustatory properties. These results could occur if gustatory neurons were intrinsically present in all ganglia, but an alternative explanation is that the tongue grafts transformed some lumbar neurons into gustatory neurons and hence neuronal plasticity is involved.

5. The fact that taste buds appeared in epithelium of dorsal and ventral tongue grafts means that under appropriate reinnervation conditions all tongue epithelium can be shown to have potential gustatory qualities. Since skin graft epithelium failed to develop taste buds an epithelial specificity must be recognized by the nerve in the tongue which is lacking in skin. The discovery of the nature and mechanism of nerve-tissue specificities could have widespread application to many problems in developmental and regeneration biology.

Proposed Course of Project: Neuronal transplant experiments will continue and the following considered:

1. Studies to determine how long neurons in histocompatible homografts can survive and function. It should be recognized that in the present studies no immunosuppressive measures were taken and the possibility of chronic neuronal rejection exists.

2. Immunosuppressive techniques will be developed to prevent immune rejection of neurons which occurs when homografts exhibit major histoincompatibility.

3. Studies will be designed to determine whether immunosuppressive agents interfere with nerve regeneration and the establishment of a functional connection.

4. Attempts will be made to grow nerve fibers from homografted neurons into the central nervous system so that a functional reflex arc can be established.

5. Morphological and physiological studies will be done to determine the ultimate success of neuronal homografts.

6. Central nervous system neuronal grafts will be attempted.

7. The antigenicity and survival of nerve homografts will be investigated to determine if nerve homografts can be used for the neurosurgical repair of traumatic peripheral nerve injuries.

8. The nature and mechanism of nerve-tissue specificities involved in taste bud formation will be pursued.

Honors and Awards: None

Publications:

Zalewski, A A.: Regeneration of taste buds after transplantation of tongue and ganglia grafts to the anterior chamber of the eye. Exp. Neurol. 35: 519-528, 1972.

Zalewski, A. A.: Trophic function of homografted neurons of Ag-B-histocompatible rats. Transplantation. 14: 618-623, 1972.

Zalewski, A. A.: Regeneration of taste buds in tongue grafts after reinnervation by neurons in transplanted lumbar sensory ganglia. Exp. Neurol. (In Press).

Zalewski, A. A.: Neuronal and tissue specificities involved in taste bud formation. Ann. N.Y. Acad. Sci. (In Press).

# ANNUAL REPORT
July 1, 1972 through June 30, 1972
Laboratory of Neural Control, Intramural Research
National Institute of Neurological Diseases and Stroke

Karl Frank, Ph.D., Chief

The Laboratory of Neural Control was established for the purpose of encouraging and carrying out research on the nervous system aimed at understanding its basic mechanisms and applying this knowledge to the development of prosthetic aids for the handicapped. It is based on the premise that enough information is presently known, or the means for discovering it are at hand, to permit the experimental building of practical devices for utilizing information transfer into and out of the central nervous system. Such long range goals include sensory aids for the blind or deaf, motor prostheses for amputees, or functional stimulation of paralyzed muscles in spinal cord injury patients and victims of stroke.

Similarly, techniques developed for neural prostheses are often valuable tools for basic research on the nervous system. Thus, in LNLC, there is a symbiotic relationship between basic research on normal neural control mechanisms and the practical application of such knowledge to relief for the neurologically handicapped.

There are a number of special problems faced by LNLC. So long as a patient is neurologically handicapped and it has been determined that the proposed neural control device will reduce that patient's handicap, there is no question of the propriety of the procedure. If the expected benefit is to some future patient, then only minor risk procedures are acceptable. In the future, it may be possible to find neural control devices which will augment the abilities of a normal person beyond normal. The ethics of such a procedure has been established and must be dealt with case by case. Inward information transfer techniques are generally less acceptable than those utilizing outward information transfer, i.e., a device by which information from the nervous system is utilized to control some feature of the environment, as in a limb prosthesis, is more acceptable than implanted electrodes, providing for stimulation of the nervous system, as in cerebellar stimulation to prevent epileptic seizures. Every modification or addition to the nervous system may have long range or even immediate social consequences which must be considered. One of the most important goals of the LNLC is the anticipation of such effects on society with the object of emphasizing those areas of research which lead to human improvement while avoiding the misuse or abuse of new and powerful techniques of neural control.

The LNLC operates in 3800 square feet of space on the 3rd and 5th floors of Building 36 on the NIH campus. There are 10 budgeted positions assigned to it, plus 2 positions borrowed from the Office of the Scientific Director. Other hiring mechanisms bring the staff to 16, including 11 Civil Servants, 3 Commissioned Officers, 1 Visiting Fellow and 1 Guest Worker. For the second year there have been no promotions in the LNLC due to restrictions against escalations in Civil Service grade point average.

## Techniques for Making Connections with the Nervous System

No method for leading out information about the activity of the nervous system has been found which is superior to the detection of the electrical signals accompanying the firing of nerve cells. Similarly, no method has been found for stimulating activity in nerve cells which is superior to the application of electrical fields through the use of electrodes. Other methods exist and are always under consideration by LNLC but so far none provides the same margin of safety between stimulation of nerve cells and irreversible damage to them. Thus, one of the most fundamental objectives of the Laboratory is the development of techniques for making chronic connections with the nervous system.

It is important to find materials, both electrical conductors and noncondutors, which can be safely implanted in animals. Among those being evaluated for biocompatibility, as well as stability of physical properties, are Parylene C, Teflon, gold and epoxy resins. Such materials all have special properties which make them useful for fabrication of stimulating or recording electrodes.

New electrode designs are being evaluated which will record the electrical activity of single nerve cells for long periods of time without destroying nearby cells. A particular problem in acute preparations is the movement of the brain relative to the electrode with vascular pulsations and respiration. In an effort to solve this problem, a new microelectrode introducer has been developed by the Biomedical Engineering and Instrumentation Branch which floats on air and rides with the movements of the brain. This device may make possible the study of single cortical nerve cell activity during human brain surgery.

Analysis and design specifications of the new Capacitor Stimulating Electrode have continued as well as its evaluation in monkeys as a chronic stimulating electrode. The special feature of this electrode is that during stimulation certain deleterious electrochemical reactions are prevented since there is no electron transfer from metal to electrolyte.

Window implants have been used to study pial blood vessel changes accompanying chronic stimulation of the cortex.

A considerable effort has been made to develop a groove electrode array for recording chronically from single nerve fibers. A successful implantation of this device is anticipated before the end of FY 73.

## Motor Control Systems Operating in the Spinal Cord

The Laboratory has continued to carry on basic neurophysiological research aimed at the elucidation of basic mechanisms of motor control operating in the spinal cord. It is here that neural signals from higher centers are translated into appropriate patterns of muscle contractions through their interactions with spinal reflex mechanisms. Information about such mechanisms is important for an understanding of the nervous system. In addition, this may lead to the design of artificial devices for performing similar integration and analysis of neural signals recorded from the brain.

This research has continued the study of the relationships of the three basic muscle fiber types to the motoneurons which innervate them. The organization of synaptic input to these cells has been studied. Motoneurons receive synaptic input, from primary muscle spindle afferents and from skin afferents among others. The synaptic potentials produced in motoneurons by primary muscle spindle activity differ in amplitude depending on the fatigue properties of the muscle fibers innervated by the motoneuron. Large synaptic potentials are produced in motoneurons supplying slowly fatiguing muscle fibers - those involved in sustained activity and the firing of spindle afferents. In contrast, skin afferent activation of motoneurons favors fast fatiguing fibers utilized in rapid unsustained muscle action, as in jumping.

Analysis of earlier data has continued to yield more detail on motor unit anatomy and innervation ratios. Muscle fiber tension produced during repetitive stimulation has been studied and muscle spindle secondary afferents have been shown to excite alpha motoneurons in decerebrated cats, an unexpected result.

The principal investigator on this project was granted the Superior Service Award for these and earlier important studies.

## Cortical Control Mechanisms

Firing patterns of cortical neurons have been studied in relationship to learned limb movements in monkeys. The objectives of this study are to determine how signals recorded from such cells can be used for the control of external devices, such as artificial limb prostheses. There are two approaches: The first utilizes the built-in organization of the nervous system, attempting to find cortical cells whose normal function can be used to control an artificial limb whose movements imitate those of the natural limb. If this can be done, the artificial limb could be substituted for the normal limb without requiring modification of normal brain behavior.

The second approach takes advantage of the adaptability of the CNS. It has already been shown that an animal can easily learn to control the firing of almost any nerve cell in the brain, and two cells can be differentially controlled. If the patterns of firing of large numbers of cells can be learned, these may be used to control a variety of external devices to which they are connected.

Studies using the first approach have identified 11 classes of firing patterns of cortical neurons related to wrist movement. They have failed to confirm the widely-accepted notion that the firing patterns generally correlate with force exerted by the limb - only a few percent show such correlation. Instead it appears that firing patterns of cortical cells may signal the muscle group to be activated during the movement. Many cells show a changing relationship to the movement. This could reflect a change in muscle pattern used by the animal to accomplish his task, or that the cell firing patterns are modified by the load experienced in the past, which is normally varied during the experiment.

Present and future experiments will explore the second approach testing adaptability of cortical neurons and will study the relation of cortical cell firing to sensory input.

## Neural Prosthesis Program

This is a collaborative program between members of the LNLC and a number of contractors at institutions outside NIH. The object of the program is to take advantage of the possibilities, as they occur, for developing prostheses for the neurologically handicapped. Three feasibility studies are currently underway: (1) a sensory prosthesis for the blind utilizing direct stimulation of the visual cortex, (2) a bladder prosthesis for spinal cord injury patients using electrical stimulation of the sacral cord, and (3) functional neuromuscular stimulation of patients with upper motor neuron lesions, through intramuscular electrodes to enable them to use some of their paralyzed muscles.

This project has grown out of an earlier project called Sensory Prosthesis for the Blind. Recognizing that most of the problems studied in that earlier feasibility study were common to the development of other neural prostheses, the objectives were broadened to take advantage of other possible applications of new basic research findings.

## Sensory Prosthesis

The University of Rochester is studying threshold changes with stimulation, stimulus pattern recognition in monkeys with arrays of intracortical electrodes and the effects of blindness on these factors.

Massachusetts General Hospital is studying afterdischarges in striate cortical neurons in relation to stimulus parameters in an attempt to determine at what rate information can be transferred through arrays of cortical electrodes. This question is of paramount importance in the feasibility of such a sensory prosthesis.

The contract with the University of Utah will be terminated pending tabulation and analysis of earlier data from stimulation of human visual cortex and from animal biomaterial studies.

Tyco Labs (now Environmental Impact Center, EIC) is continuing studies of electrochemical reactions occurring when stimulating currents are passed across the electrode-electrolyte boundary in an effort to find the best conditions for electrical stimulation without damage to electrodes or nervous tissues.

The Huntington Institute of Applied Medical Research is continuing its studies on the histopathological effects of chronic stimulation through implanted electrodes in cats. It is also concentrating on new methods to limit the spread of stimulating currents.

The University of Florida is using powerful metallurgical tools for studying corrosion of electrode materials from electrical currents.

## Bladder Prosthesis

Contracts are just beginning with Duke University and the University of California at San Francisco to develop means for evacuation of the bladder in patients with spinal cord injuries through stimulation of the sacral spinal cord. The major problem appears to be the need to stimulate contraction of the detrusor muscle of the bladder wall, while simultaneously inhibiting the contraction of the sphincters limiting outflow of urine. These contracts provide a well-justified reason for studying the problems of pattern stimulation through arrays of electrodes chronically implanted in the CNS. Preliminary results appear to be quite promising.

## Functional Neuromuscular Stimulation

Case Western Reserve University, by sequential stimulation through arrays of implanted intramuscular electrodes in patients with upper motor neuron lesions, has been able to achieve smooth proportional control of thumb and finger grasp in one patient. They have results in animals suggesting increased resistance to fatigue, slowing of contraction time and reduction of peak force following long-term stimulation of muscles through their motor nerves. Histochemical changes in chronically stimulated muscle fibers suggest an increased capacity for oxidative metabolism, as seen in slowly fatiguing muscle types. The scope of this contract has been increased to include more complex patterns of functional neuromuscular control, as well as further studies of changes in muscle resulting from its chronic stimulation.

Fundamental to all the neural prostheses currently under study or anticipated in the future is the development of safe, effective means of selectively exciting or inhibiting small populations of neurons.

Serial No. NDS(I)-69 LNLC/OC 168
1. Laboratory of Neural Contr
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Motor Control Systems in the Spinal Cord

Previous Serial Number: Same

Principal Investigator: Robert E. Burke, M.D.

Other Investigators: William Z. Rymer, M.D.
John V. Walsh, M.D.
David F. Noble, Ph.D.
George M. Dold

Cooperating Units: None

Man Years:
Total: 3.5
Professional: 3.1
Other: 0.4

Project Description:

Objective: The objective of this project is to provide information in the following related areas: (a) concerning th interrelation between the intrinsic properties of alpha motoneurons and the physiological, morphological and histochemical characteristics of the muscle fibers innervated by the investigated motoneurons; (b) concerning the patterns of use of motor units of different types during a variety of movements in anesthetized and in alert, freely moving animals; (c) concerning the organization of synaptic input to alpha motoneurons fr segmental reflex and descending systems; and (d) concerning th interaction of segmental and descending systems in the control of information flow in spinal segmental motor mechanisms.

Methods Employed: Anesthetized cats have been used in acu experiments involving intracellular microelectrode recording ar stimulation techniques. Special methods permit recording the mechanical responses of single motor units in cat hindlimb muscles following intracellular stimulation of their motoneuro We are exploring several techniques whereby the pattern of activity of single motor units can be recorded in awake, freel moving animals and which also permit subsequent physiological characterization of the same motor units. These methods invol sequential chronic and acute experiments on the same animal. The response and central effects of muscle spindle secondary (group II) afferents is being investigated in anesthetized or decerebrate cats, using mechanical muscle stimulation and conventional techniques for recording resultant motoneuron outputs.

Major Findings:

A. Organization of synaptic input to motor units of defined type: From previous work in this laboratory, it is known that motor units in the heterogeneous gastrocnemius muscle of the cat hindlimb fall into 3 major categories on the basis of differences in physiological properties. A precise relation exists between these categories and the classes of muscle fibers in the same muscle, based on histochemical and morphological characteristics. Building upon these results, we have examined the organization of synaptic input from a variety of sources impinging on alpha motoneurons which innervate defined types of muscle fibers. Some input sources, notably the primary muscle spindle afferents which monosynaptically excite motoneurons, produce postsynaptic potentials (PSPs) of largest amplitude in type S motor units (slowly contracting small tension, resistant to fatigue; muscle units dependent on aerobic glycolysis), in general somewhat less large in type FR units (fast contracting but also resistant to fatigue, moderate tension; muscle units with both aerobic and anaerobic capacities), and smallest among type FF units (fast contracting, high tension, very sensitive to fatigue; muscle units dependent on anaerobic glycolysis). Activation of the motor unit pool via input systems organized in this way produces firing in small, slow type S units and, with increasing input, in faster, medium tension FR units. The fatigue resistant character of these unit types has obvious advantages in sustained activity, such as walking and running which is believed to involve activity of muscle spindle afferents.

A qualitatively different pattern of input organization has been found for other systems, including a polysynaptic system activated by skin afferents. Such systems produce predominant excitatory PSPs in both FF and FR unit motoneurons, and predominant inhibitory PSPs in motoneurons of type S units. Activation of the motor unit pool by such a system would produce a reversal of the above recruitment pattern among the motor units, and it can be suggested that such reversal may be advantageous in situations requiring very high tension, rapid muscle action which is not necessarily sustained for prolonged periods (e.g. sudden accelerations in jumping).

B. Motor unit anatomy and innervations ratios: During this year, material available from previous work has been analyzed to define the distribution of muscle fibers belonging to single motor units within the cat gastrocnemius and soleus muscles. The unit territories are surprisingly widespread, with considerable overlap between the territories of different units. In cat gastrocnemius, a single alpha motoneuron innervates between 400 to 800 muscle fibers on the average, irrespective of motor unit type. Specific tension output estimated for type S unit fibers (0.6 $kg/cm^2$) is less than one-half that of FF and FR unit fibers (1.5 to 2.4 $kg/cm^2$). The innervation ratio in soleus is smaller (about 150 fibers per motor unit).

C. Tension production in single motor units during repetitive firing: Mechanical tension production of single motor units has been analyzed with respect to the question of non-linear behavior of the fiber contractile element during repetitive activation. It has been found that non-linearities in development of the fiber active state appear to be optimized in each class of motor units to match the preferred firing frequencies at which the various classes of units are known to fire.

D. Analysis of the central effects of muscle spindle secondary afferents: The effect of muscle spindle secondary afferent discharge on alpha motoneurons is being studied using both ventral root and intracellular recording techniques. The results to date suggest that spindle secondaries may be excitatory to extensor alpha motoneurons under certain conditions (e.g., the decerebrate state), in contract to the commonly accepted inhibitory role for these afferents. The mechanism of motoneuron excitation is unclear and studies are underway to examine possible contributions from presynaptic and disinhibitory effects.

E. Collaborative work: Dr. R. E. Burke spent 11 weeks (February-April, 1972) as an invited visiting scientist in the Department of Physiology, Center for Research and Advanced Studies, National Politecnic Institute, Mexico City. There he worked with Dr. P. Rudomin on questions of presynaptic control of information flow in ensembles of muscle spindle primary afferents to alpha motoneurons. Computer analysis of data obtained are still in progress.

During this fiscal year, Dr. Burke has spent a significant fraction of time writing an extensive review chapter for the Handbook of Physiology at the invitation of the American Physiological Society.

Significance to Biomedical Research and the Program of the Institute: The problem of motor control in the intact animal involves consideration of the functioning of motor units as individual entities, active in asynchronous patterns. Much of the present work has been designed to further our understanding of the input-output relations of motor units, considered on the basis of single units rather than whole muscles or muscle nuclei. It can be expected that information regarding the organization of synaptic input to different groups of motor units within the same muscle will give a better picture of how the central nervous system may utilize these units during movements of various sorts. In addition, knowledge of the mechanical output characteristics of the same motor units permits inferences about the ultimate result of patterns of motor unit discharge in terms of muscle tension output. The motor unit has long been recognized as the ultimate target of integrative mechanisms in the motor system. The aim of this project is to contribute to a detailed understanding of the mechanisms, particularly those in and projecting to the spinal cord, which operate to control motor behavior.

Proposed Course of Project: During FY 1974, the ongoing analysis of synaptic organization patterns will be continued, with particular emphasis on examination of neuronal systems descending from supraspinal centers to the spinal segments. A series of acute experiments is planned to study the patterns of motor unit recruitment to peripheral and supraspinal stimulation. The usage patterns of defined types of motor units in intact, moving animals will be studied, reactivating a project begun several years ago but held in abeyance recently because of technical problems. Preliminary experiments are underway which are designed to examine the effect of increased output demand, and muscle hypertrophy, on the characteristics of single motor units. Finally, the work on spindle secondary afferents will be continued to anticipated completion.

Honors and Awards: DHEW Superior Service Award to Dr. R. E. Burke, June, 1972.

Publications: Burke, R.E.: On the central nervous system control of fast and slow twitch motor units. In Desmedt, J.E. (ed.): New Developments in Electromyography and Clinical Neurophysiology. Vol. 1, Basel, Switzerland, Karger:Basel, 1973, pp. 69-94.

Dold, G.M. and Burke, R.E.: A joystick operated microforge for fabrication of glass micropipette electrodes. Electroencephalogr. Clin. Neurophysiol. 33: 232-235, 1972.

Burke, R.E., Tsairis, P., Levine, D.N., Zajac, F.E., and Engel, W.K.: Direct correlation of physiological and histochemical characteristics in motor units of cat triceps surae muscle. In Desmedt, J.E. (ed.): _New Developments in Electromyography and Clinical Neurophysiology_. Vol. 3, Basel, Switzerland, Karger:Basel, 1973, pp. 23-30.

Granit, R. and Burke, R.E.: The control of movement and posture. _Brain Res_. 53: 1-28, 1973.

Burke, R.E. and Tsairis, P.: The correlation of physiological properties with histochemical characteristics in single muscle units. _Ann. NY Acad. Sci_. In press.

Serial No. NDS(I)-69 LNLC/OC 1687
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Techniques for Making Connections with the nervous system

Previous serial Number: Same

Principal Investigator: Frederick T. Hambrecht, M.D.

Other Investigators: Martin Bak
Karl Frank, Ph.D.
Seth Goldstein, Ph.D.
David Guyton, M.D.
George Laird
William Marks, Ph.D.
W. Zev Rymer, M.D.
Edward Schmidt, Ph.D.

Cooperating Unit: Division of Research Services - Biomedical Engineering and Instrumentation Branch
Serial No. DRS-BEIB-12

Man Years:

| | |
|---|---|
| Total: | 4.9 |
| Professional: | 2.7 |
| Other: | 2.2 |

Project Description:

Objectives: Each of the major goals of the Laboratory of Neural Control requires the development of techniques for making chronic connections to the nervous system for the purpose of selective recording and stimulation. Many parallel channels of information are required from individual nerve cells or small groups of cells of similar function. In accomplishing these objectives, it is necessary to: (a) develop new types of chronically implantable electrodes, (b) make connections between such electrodes and recording or stimulating devices outside the body, and (c) analyze the recordings from such electrodes so as to separate the signals from individual cells.

Methods Employed:

A. Materials suited for biological implantation. During the current period, several new materials have been chronically implanted in animals and evaluated for stability of their physical properties and their biocompatibility. These include Parylene C, Teflon, gold and epoxy resins. Evaluation of cyanoacrylate adhesives, platinum-iridium alloys and sintered tantalum-tantalum oxide has continued from last year.

B. Designs for a chronic recording intracortical microelectrode. Following the successful demonstration of prototype intracortical microelectrodes for chronic recording, a second generation "hatpin" electrode was designed. These electrodes have a sharpened iridium shaft welded to a flexible gold lead out wire. The weld is covered with a ball of epoxy and the entire assembly is coated with Parylene C except for the top of the sharpened shaft. The advantages of the "hatpin" configuration over "tack" electrodes include easier insertion, lower electrical capacity and the potential for higher density electrode arrays. A prefabricated acrylic chamber to cover the craniotomy site has also been evaluated and should shorten the time required during insertion of the electrodes. Two monkeys have had hatpin electrodes inserted; 12 electrodes in the first monkey, 10 in the second. Histological studies of the tissue surrounding these electrodes are planned.

C. Microelectrode introducer capable of tracking the moving brain. An electrode introducer for use in obtaining single unit recording in the human brain has been fabricated. The electrode is mounted in a rider tube and is positioned by a 160 turns per inch lead screw. The rider tube is supported without friction by two air journal bearings allowing it and the electrode to freely follow the pulsatile movement of the exposed cortex. The weight of the rider tube is automatically compensated over a wide range of orientation angles. A series of eight thrust bearings acting upon fins attached to the rider tube and lead screw allow electrode depth to be varied without interfering with the tracking of pulsatile cortical movement.

D. Capacitor stimulating electrode. Work has continued on the "capacitor electrode," a porous tantalum disk completely insulated by a thin layer of tantalum pentoxide. The electrode is designed to stimulate neural tissue without generating harmful electrochemical products. Specifications for electrode construction were derived from mathematical modeling, and electrodes with these specifications were obtained from a capacitor manufacturer. These electrodes are 1.0 mm in diameter by 0.25 mm thick, with a capacitance of 1.4 uf and a working voltage of 4 volts. A technique was devised to shield the back and sides of each disk with a plastic cap of vapor-deposited Parylene C. Stability of the electrodes was studied by long-term continuous electrical pulsing in saline and in human plasma. An in vitro pH test was devised to detect minute net pH changes in the vicinity of stimulating electrodes, and this test was used in comparing the capacitor electrodes with capacitively-coupled platinum electrodes. Capacitor and platinum electrodes were also implanted on the motor cortex of a rhesus monkey to study the effects of chronic stimulation. Twenty stimulation sessions averaging five hours each were performed over a six month period, while monitoring the electrical characteristics of the electrodes and following the threshold for motor response at each electrode.

E. Effects of chronic stimulation on the pial vasculature. Plastic (Lucite) windows, 2 cm in diameter, were constructed for implantation through the scalp, skull, and dura of rhesus monkeys such that the pial vasculature could be viewed under closed conditions on a long-term basis. Incorporated within each window was a saline-filled chamber containing a large-surface, capacitively-coupled platinum electrode. A pore 1.0 mm in diameter led from the saline-filled chamber to the pial surface. The purpose of this arrangement was to provide a "pore electrode" essentially free of electrochemical effects, and transparent so that the pial vasculature could be viewed and photographed directly beneath the "electrode." Two of these chambers were implanted under sterile conditions over the motor cortex of a rhesus monkey. Successful results were only obtained in the five or six hour period immediately following each implantation, for tissue reaction soon clouded the cortical details beneath each chamber, with no significant clearing on three weeks of observation.

F. Groove electrode arrays for chronic recording from nerve fibers. Electrode arrays consisting of 3 to 6 grooves, each 5 mm long and 50 to 150 microns wide and deep, in a sandwich of epoxy and Teflon have been fabricated. Continuous nerve filaments split off of spinal roots are drawn into the grooves by the hydrophilic saline in the grooves. A shunt path between the grooves is prevented by the hydrophobic properties of Teflon. An insulating cover is then placed over the grooves. Recordings are made from electrical contacts at the midpoints of each groove.

Major Findings: The tantalum capacitor electrode is stable during long-term in vitro testing in saline and in plasma. Local pH studies demonstrate a clear advantage of the capacitor stimulating electrodes over similarly shaped platinum electrodes by the absence of detectable pH changes in the vicinity of the capacitor electrodes.

The circulation through the pial vasculature is affected by electrical stimulation in the absence of electrochemical effects at levels sufficient to excite cortical neurons. Limited observations prevent more definitive statements at this time.

Single unit activity has been recorded from nerve fibers in groove electrode arrays. Waveforms recorded from healthy fibers are triphasic. Using such arrays, the activity of 10 to 30 separate dorsal root fibers have been monitored simultaneously, representing fast and slow adapting cutaneous receptors, hair movement receptors and muscle spindles.

Significance to Biomedical Research and the Program of the Institute: The successful development of techniques for recording signals from the nervous system and means of selectively controlling the activity of small populations of neurons is mandatory for success of the Laboratory objectives. Such developments are also of value to the neurophysiological research of other laboratories, including those working on prostheses for the neurologically handicapped.

Proposed Course of Project: All of the projects discussed need further development and work will continue on them. In the near future, a project will be initiated to attempt to selectively and reversibly inhibit the electrical activity of small groups of neurons. Further cooperation with contractors in the Neural Prosthesis Program is anticipated.

Honors and Awards: None

Publications: Guyton, D.L. and Hambrecht, F.T.: Capacitor electrode: Stimulates nerve or muscle without oxidation-reduction reactions. Science, 1973, in press.

Salcman, M.: Long-term recording from single cortical units. In Functional Neuromuscular Stimulation. Washington, D.C., National Academy of Sciences, 1972, pp. 125-132.

Guyton, D.L.: The capacitor-stimulating electrode. In Functional Nueromuscular Stimulation. Washington, D.C., National Academy of Sciences, 1972, pp. 135-142.

Salcman, M. and Bak, M.: Design, fabrication and in vivo behavior of chronic recording intracortical microelectrodes. IEEE Trans. BME 20: 4, 1973.

Goldstein, S. and Salcman, M.: Mechanical factors in the design of chronic recording intracortical microelectrodes. IEEE Trans. BME 20: 4, 1973.

Serial No. NDS(I)-69 LNLC/OC 1688
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cortical Mechanisms of Voluntary Motor Control

Previous Serial Number: Same

Principal Investigator: Edward M. Schmidt, Ph.D.

Other Investigators: Martin Bak
Kathleen Davis
George Dold
Karl Frank, Ph.D.
Herbert Lansdell, Ph.D.
Joan McIntosh

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 2.9 |
| Professional: | 1.0 |
| Other: | 1.9 |

Project Description:

Objectives: The major goal of this project is to study the feasibility of using cortical neurons for the control of external devices such as a prosthesis. This involves the study of relationships between the firing patterns of cortical neurons and the measurable parameters associated with simple movements. Our aim is to determine whether it is possible to extract enough information from the spike trains of small sets of cortical neurons to be able to predict the time course and amplitude of various motor response measurements. This requires a careful study of the types of cells to be found in the motor cortex; their frequency of occurrence, location and specific function. At the same time, we are anxious to determine how much control an individual has over the firing patterns of these cortical cells. If an individual can influence the firing rate of specific neurons, the chances for building a centrally controlled prosthesis are markedly improved.

Methods Employed: Monkeys are trained to move a small handle which is controlled by a torque motor under servo control. The force, position and velocity of the handle are continuously monitored. By operating the servo mechanism in various modes, all mechanical properties of the load (inertia, viscous friction, and spring constant) are under the immediate control of the investigator and loads can suddenly be altered while monitoring cortical cell firing and motor performance. Simultaneous extracellular recordings are obtained from the motor cortex using the Evarts chronic microelectrode recording system, and when multiple cells occur on a single electrode, the firing patterns of individual cells are separated and counted in real time. All data can be digitized and pre-processed at the time of the experiment.

Major Findings:

1. It has been widely accepted that neurons in the motor cortex are correlated with the force exerted by a limb, and not with the position of the limb. Our current experiments, specifically designed to analyze the force relationship, have not found any cortical cells related to wrist movement that show a close relationship to the force produced by the limb.

We have been able to classify the firing patterns of task related cortical cells into 11 separate categories. The firing pattern of a particular cell may be related to flexion, extension, or both. In addition, the cell may be active in one phase of the movement, fire briefly prior to the movement, stop background firing before a movement, or fire continuously with the rate modified by the movement. One function of cortical cell firing patterns appears to us to be the signaling of the muscle group to be activated during the movement. Knowledge of the normal firing patterns of cortical cells will allow us to design equipment to utilize these signals for control of external devices such as artificial limbs.

2. The firing patterns of a number of task-related cortical cells have shown a plastic relationship to the movements. There are two possible explanations for this finding. The first is that the animal changes the manner in which he performs the task and the cortical cells reflect this change. The second possibility is that firing patterns are modified depending on the loads experienced in the past. If the latter situation is true, it should be possible for the monkey to modify his cell firing pattern to control external devices.

3. We have found that a monkey can be conditioned to control the firing rate of an individual cell in the motor cortex. The next question to answer is: How many cells can the monkey simultaneously and independently control? With this goal in mind, we have been attempting to chronically record from the

same group of single cells for long periods of time. Improvements in microelectrodes and implantation techniques are yielding prolonged recording sessions. In the near future sufficiently long recording sessions are foreseen to adequately answer our questions.

Significance to Biomedical Research and the Program of the Institute: Through our studies we are obtaining a better understanding of the function of the motor cortex and the types of control signals we can obtain. We are quite optimistic that long-term chronic recordings can be obtained from the motor cortex and these signals used to control the stimulation of paralyzed muscles or control a prosthetic device.

Proposed Course of Project: Work will continue in the area of making long-term chronic recordings from cells of the motor cortex. Attempts will be made to use the firing patterns of these cells to control a device such as an artificial limb.

Another area of interest is how the firing patterns of cortical cells are modified after bilateral rhizotomy. This type of preparation would approach the conditions present in quadriplegics and amputees and would tell us if the firing patterns of motor cortex cells in normal monkeys were different from those in rhizotomised animals. In these same experiments we will also determine if anatomical changes occur in the organization of the motor cortex when sensory input from the limbs is removed. These experiments should bring us closer to the problems we will face when electrodes are implanted in man for the control of artificial limbs or activation of paralyzed muscles.

Honors and Awards: None

Publications: Schmidt, E.M.: Electronically controlled load for monkey manipulandum. Electroencephalogr. and Clin. Neurophysiol. 35: 95-97, 1973.

Serial No. NDS(I)-71 LNLC/OC 1887
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Microstimulation of the Nervous System

Previous Serial Number: Same

Principal Investigator: Edward M. Schmidt, Ph.D.

Other Investigators: None

Cooperating Unit: None

Man Years:

| | |
|---|---|
| Total: | 0 |
| Professional: | 0 |
| Other: | 0 |

Project Description:

This project has been terminated.

Serial No. NDS(I)-71 LNLC/OC 1916
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Feasibility of a Sensory Prosthesis for the Blind

During FY 1973 this project was incorporated with Serial No. NDS(I)-73 LNLC/OC 2015.

Serial No. NDS(I)-73 LNLC/OC 2015
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neural Prosthesis Program

Previous Serial Number: New and incorporating Serial No. NDS(I)-71 LNLC/OC 1916

Project Officers: Frederick T. Hambrecht, M.D.
Karl Frank, Ph.D.

Other Investigators: David L. Guyton, M.D.
Herbert C. Lansdell, Ph.D.
Edward M. Schmidt, Ph.D.

Cooperating Unit: None

Man Years:

| | |
|---|---|
| Total: | 3.2 |
| Professional: | 2.4 |
| Other: | 0.8 |

Project Description:

Objectives: The possibility of using recent advances in the basic neurosciences, material sciences and microelectronics for the development of prostheses for the neurologically handicapped is being investigated. In particular, the feasibility of a sensory prosthesis for the blind utilizing direct electrical stimulation of the visual cortex, a bladder prosthesis for patients with neurogenic bladders utilizing electrical stimulation of the sacral spinal cord, and functional neuromuscular stimulation with intramuscular electrodes in patients with upper motor neuron lesions are being studied.

Methods Employed: The feasibility studies are being carried out primarily through contracts with nine institutions.

Functional Neuromuscular Stimulation: Case Western Reserve University has implanted intramuscular electrodes in paralyzed patients and is studying the degree of proportional control that can be achieved. Sequential stimulation through multiple electrodes is utilized to provide smooth muscle contractions and to reduce fatigue. They are also studying the effects of electrical stimulation on the physiological and histological properties of muscles in both normal and spinal animals.

Bladder Evacuation: Both Duke University and the University of California at San Francisco are stimulating the spinal cords of both normal and spinal animals to determine if cell populations exist in the cord which can be selectively stimulated to cause bladder detrusor contraction with simultaneous sphincter relaxation.

Sensory Prosthesis: The University of Rochester, using sighted and blinded rhesus monkeys, is studying methods of optimizing information input to the visual cortex, survival of electrical excitability during long-term stimulation, and the effects of blindness on these factors.

The Massachusetts General Hospital is investigating the relationships between stimulus parameters and afterdischarges following electrical stimulation of the visual cortex in animals. They are also studying the effects of changes in electrode parameters and electrode size by stimulating visual cortex of human subjects who require occipital craniotomies.

The University of Utah is analyzing and tabulating their data, both from human visual cortex stimulation and from animal biomaterial implantation. Following this, their contract will be terminated.

Basic Studies: Tyco Laboratories is determining the electrochemical reactions at the electrode-electrolyte junction during the passage of current and methods of identifying and minimizing possible toxic byproducts.

The Huntington Institute of Applied Medical Research is concentrating on developing methods of confining current spread during electrical stimulation. They are also continuing their series of chronically stimulated animals with subsequent histopathological examination of surrounding tissue. Human stimulation experiments have been temporarily abandoned pending results of further animal experiments.

The University of Florida is performing long-term in vitro tests of potential electrode materials using scanning electron microscopy and atomic absorption spectrophotometry to evaluate corrosion of these electrodes during electrical current passage. Promising materials are used as chronic stimulating electrodes in animals with subsequent histopathological study of surrounding tissues.

The Project Officers have coordinated some of their research activities at NIH with those of the contractors and have organized two workshops, one on functional neuromuscular stimulation and one on sensory prostheses.

Major Findings: Cytoarchitectonic studies of the human visual cortex indicate that only about 30% of the area of the striate cortex is superficial and exposed while the remainder is deep in sulci or fissures, a typical value of exposed area on one hemisphere is 9 $cm^2$.

Continuous electrical stimulation of the visual cortex in macaques for periods greater than two hours produces an elevation of threshold for detection by the animal whose magnitude and permanence depends on the type and intensity of stimulation.

The threshold for afterdischarges in the cat striate cortex following stimulation of the surface is more closely related to total current delivered than to local current density at the electrode-tissue interface. It appears that stimulus parameters can be adjusted such that the latency for visual neuron firing can be reduced while still preventing afterdischarges. The afterdischarge threshold is higher for short, repetitive trains than for continuous stimulation.

Histopathological examination of cat brains following chronic stimulation for 20 hours over four to five month periods with biphasic, capacitively coupled stimuli and parameters suitable for producing phosphenes in humans has revealed no significant tissue damage. However, similar stimulation with direct coupled stimuli results in severe neuronal destruction with a chronic inflammatory reaction.

Electrochemical studies of platinum electrodes in simulated cerebrospinal fluid indicates that at least 175$\mu$ coulombs/real $cm^2$/pulse can be passed without producing solution species. This is important because byproducts of electrical stimulation must be in solution to produce toxicity.

Five metals have now been classified on the basis of their resistance to corrosion during current passage into simulated cerebrospinal fluid. In order of decreasing resistance, they are Rh, Pt, Pt-10% Ir, Pt-10% Rh, Au. However, preliminary in vivo testing of Rh indicates that it may be toxic.

Long-term stimulation of muscles in animals has demonstrated increased resistance to fatigue, slowing of contraction time and reduction of peak force compared with unstimulated, contralateral control muscles. Histochemical evaluation of the stimulated muscles shows an increased capacity for oxidative metabolism. Similar stimulation in human muscle paralyzed from upper motor neuron lesions and atrophied from disuse results in increased resistance to fatigue and increased force of contraction. Sequential stimulation through three electrodes placed in the forearm flexor muscles of a paralyzed patient resulted in smooth flexion of fingers 2-4, with pronation of the forearm, which could be proportionally controlled by the patient. This has been used by the patient for effective and quick grasp when the thumb is braced.

Significance to Biomedical Research and the Program of the Institute: As these feasibility studies continue, the possibility of developing truly useful neural prostheses for the neurologically handicapped becomes more promising. The results of these studies are already proving useful to current neurosurgical techniques involving electrical stimulation and manufacturers of equipment for such stimulation have utilized results from these studies for the design of their equipment. Many of the findings will be applicable to other future devices, such as auditory prostheses for the deaf and electrical stimulators of the cerebellum for relief of intractable epilepsy.

Proposed Course of Project: Further studies of the type indicated above are required, as well as investigation of the feasibility of new types of neural prostheses as basic research suggests possible modes of operation. Fundamental to all the neural prostheses presently being investigated is the further development of safe, effective means of selectively exciting or inhibiting small populations of neurons.

Honors and Awards: None

Publications: Hambrecht, F. T.: Neural control aspects of functional neuromuscular stimulation. In Functional Neuromuscular Stimulation. Washington, D.C., National Academy of Sciences, 1972, pp. 111-114.

Hambrecht, F.T.: Stimulation of human visual cortex. IEEE Interconference Record, 1973. In press.

Hambrecht, F.T.: Visual prostheses: Theoretical objectives, present status and future possibilities. Proceedings of the Houston Neurological Symposium, 1973. In press.

Hambrecht, F.T.: The current status of visual prostheses. Am. J. Opthalmol. In press.

# ANNUAL REPORT
## July 1, 1972 through June 30, 1973
## Laboratory of Neurophysiology, Intramural Research
## National Institute of Neurological Diseases and Stroke

M.G.F. Fuortes, M.D., Chief

Major changes in the staff of the Laboratory of Neurophysiology have occurred during the present fiscal year. Fortunately, the losses due to transfer of scientists to other institutions or to reduction in the number of positions have been largely compensated by the influx of visiting scientists, fellows and guest workers.

Members of the staff gave a course of neurophysiology at the Washington Medical Center, delivered invited lectures in the U.S. and abroad and participated as editors and contributors to the publication of a Handbook of Sensory Physiology.

Collaboration with other Institutes has been established or continued. It has been especially active with the C.N.R. in Italy; the Physiological Laboratory of Cambridge, England; the University of Colorado and the NICHD.

The major topics of the research performed during the year include: microspectrophotometry; invertebrate neurophysiology; retinal organization; and cytology. These studies resulted in the publication of eighteen papers.

Microspectrophotometry. Satisfactory measurements of pigment absorption were obtained in single rods (frog) and single cones (goldfish). Rod pigments have absorption maxima at 502nm and 380nm, with chromophore orientation perpendicular to the axis of the outer segment. Specific optical density was .017±.0015/μm. Cone pigments have maxima at 455±3nm, 530±3nm and 625±5nm. Cones containing pigments of different colors are appreciably different in their morphology. Specific optical density is somewhat less than in rods: .0124±.0015/μm in the average. The results suggest that the concentration of the visual pigment is the same in frog rods and goldfish cones. Molar extinction of the blue and green cone pigments is less than for the red pigment.

Neurophysiology. Work on the nervous system of invertebrates has been continued. Voltage-clamp studies on snail neurons which present rhythmical bursts of activity ("pacemaker" neurons) led to the identification of the membrane mechanisms underlying this type of rhythmicity. It seems possible that the same general processes underlie other types of rhythmical activities (heart and smooth muscles).

Comparative studies on photoreceptors have revealed that the electrical responses (voltages and conductances) differ in different animals. The kinetics of the responses, however, have many common properties and can be interpreted assuming that in all cases, the electrical response to light is mediated by the production of a chemical substance capable of reacting with the membrane.

Interesting results have been obtained in the studies of interactions in the simple nervous system of molluscs. It was found that receptors responding to accelleration (there are twelve hair cells of this type in each of the two statocysts of Hermissenda) interact with each other: the cells which are excited by accelleration in one direction inhibit those which respond to accelleration in the opposite direction. In addition, hair cell activity can inhibit the responses to light of photoreceptors in the ipsilateral eye and conversely excitation of the eye can modulate the activity of the hair cells.

Retina. The study of retinal organization continues to be one of the major efforts of the Laboratory. Morphological studies have now led to a satisfactory description of the synaptic contacts and electrical junctions between cells. Physiological work has been successful in establishing one major function of horizontal cells: to respond to the activity of large numbers of receptors and to send signals back to them. By this mechanism, the response of each receptor changes as a function of the pattern and colors of the light falling on the whole retina. The feedback signal originated by the horizontal cells reaches not only the receptors which have been exposed to light but also those which have remained in darkness. In the first case, the feedback adds a depolarizing wave to the hyperpolarizing direct response of the receptor; in the second case it evokes a pure depolarization of the receptor. Thus, receptor responses may be of opposite polarity, for different distributions of the retinal illumination.

Cytology. Mitotic activity and DNA synthesis were measured in the epithelium of the crystalline lens of rats. It was found that isoproterenol decreases mitotic activity. Dibutyryl and monobutyryl cyclic AMP were also effective in suppressing mitosis. The mode of action, however, is different for the two types of drugs.

Serial No.:NDS(I)-65 LNP/SP-1239
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Photoreceptors in the Limulus Eyes

Previous Serial Number: Same

Principal Investigator: Thomas G. Smith, Jr., M.D.

Other Investigator: George C. Murray, Ph.D.[1]

Cooperating Units: [1]Office of the Associate Director, C&FR/NINDS

Man Years:

| | |
|---|---|
| Total: | 0.6 |
| Professional: | 0.5 |
| Other: | 0.1 |

Project Description:

Objectives: The objective of this research is to gain insight into the means by which light is transduced into an electrical current in photoreceptors. The intracellularly recorded potentials of the photoreceptor cells of the horseshoe crab, Limulus polyphemus, are the object of study.

Methods Employed: Responses are recorded with intracellular microelectrodes and analyzed under a variety of experimental conditions. Voltage clamp of the membrane potential is the main method currently employed in our experiments. Using this technique, the role of visual pigments, of photoreceptors cell membrane and of various ions in photoreception can be studied.

Major Findings: The investigations have been concerned with an analysis of the membrane properties of Limulus photoreceptors in the dark, in steady light and after their treatment with metabolic inhibitors (strophanthidin).

Further analysis of the voltage and time-dependent conductances reported in the previous report have been carried out. The results have been compared to various models of the action of light and metabolic inhibitors (glycosides) on these conductances. No model involving conductance changes to only one ionic species has been found which fits the data. Thus, conductance changes involving two or more ionic species must be postulated to account for the steady state changes induced by light and by the glycosides. As yet the data are not sufficient to specify in detail the contribution of the various ions involved.

Significance to Bio-medical Research and the Program of the Institute: The results add further confirmation that those model of photoreceptor function based either on linear and time-independent processes or on conductance changes involving only one ionic species are inadequate to account for the properties of *Limulus* ventral eye photoreceptors and for the action of light and of the cardiac glycosides on these cells. Moreover, hypotheses which propose that light leads to an increase of membrane conductance are not consistent with present results on the steady-state part of the light response.

Proposed Course of the Project: It is proposed to continue the study of membrane properties of *Limulus* photoreceptors by the voltage-clamp techniques. These studies will also include the investigation of the effects of altered ionic environment and of other metabolic inhibitors on the functions of photoreceptors. Hopefully such experiments will provide information on which ionic species carry and regulate the current through the time-independent and the various time-dependent conductances

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-69 LNP/SP-1690
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Rapid High Resolution Microspectrophotometry in Visual Cells

Previous Serial Number: Same

Principal Investigator: George C. Murray, Ph.D.[1]

Other Investigators: Franco Malerba, Ph.D.
Thomas G. Smith, Jr., M.D.
Theodore Colburn, Ph.D.[2]
Ferenc Harosi, Ph.D.

Cooperating Units: [1]Office of Associate Director, C&FR/NINDS
[2]Section on Technical Development, NIMH-NINDS

Man Years:

| | |
|---|---|
| Total: | 1.6 |
| Professional: | 1.5 |
| Other: | 0.1 |

Project Description:

Objectives: The objective of this research is the development of rapid scanning microspectrophotometric techniques for investigation of the chemical kinetics within single excitable cells. Although these techniques are being developed to study the molecular steps coupling photoexcitation of visual pigments to the excitation of the electrical response of the cells, their more general application to cellular physiology will also be considered.

Methods Employed: The microspectrophotometer samples transmittance at spectral wave bands between 400 and 600 nm, either sequentially or in random order, at rates of 33 microseconds per sample point. The system uses a rapid scan monochromator in which the entrance slit has been replaced by a cathode ray tube face.

Major Findings: The prototype system has been replaced by the circuitry necessary to develop the final system. These replacements include 1) a new cathode ray tube, 2) new power supplies and 3) better amplifiers and signal control units. The new CRT was specially built for this instrument by RCA and contains a mixture of three phosphors that will provide the full spectrum of wavelengths required. The new power supplies will provide better stability and control of the light beam. And, finally, the new amplifiers and signal control units provide a wider dynamic range and faster operation of the unit than were possible in the prototype system.

Significance to Biomedical Research and the Program of the Institute: This project should provide the specific instrumentation and techniques for the measurement and analyses of the transduction steps which couple the stimulus to the electrical changes in the excitable membrane of photoreceptors. It will also provide a useful tool for similar study of other molecular systems functioning within living cells, for which an increasing need has developed.

Proposed Course of the Project: Once the final instrument is completed, it will be tested out by using objects of known spectral composition in order to determine the optimum operating parameters of the system. Subsequently, we will begin the investigation of visual photoreceptors and some vital staining of intracellular components in photoreceptors and other cells.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-69 LNP/SP-1690
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Rapid High Resolution Microspectrophotometry in Visual Cells

Previous Serial Number: Same

Principal Investigator: George C. Murray, Ph.D.[1]

Other Investigators:
Franco Malerba, Ph.D.
Thomas G. Smith, Jr., M.D.
Theodore Colburn, Ph.D.[2]
Ferenc Harosi, Ph.D.

Cooperating Units:
[1]Office of Associate Director, C&FR/NINDS
[2]Section on Technical Development, NIMH-NINDS

Man Years:

| | |
|---|---|
| Total: | 1.6 |
| Professional: | 1.5 |
| Other: | 0.1 |

Project Description:

Objectives: The objective of this research is the development of rapid scanning microspectrophotometric techniques for investigation of the chemical kinetics within single excitable cells. Although these techniques are being developed to study the molecular steps coupling photoexcitation of visual pigments to the excitation of the electrical response of the cells, their more general application to cellular physiology will also be considered.

Methods Employed: The microspectrophotometer samples transmittance at spectral wave bands between 400 and 600 nm, either sequentially or in random order, at rates of 33 microseconds per sample point. The system uses a rapid scan monochromator in which the entrance slit has been replaced by a cathode ray tube face.

Major Findings: The prototype system has been replaced by the circuitry necessary to develop the final system. These replacements include 1) a new cathode ray tube, 2) new power supplies and 3) better amplifiers and signal control units. The new CRT was specially built for this instrument by RCA and contains a mixture of three phosphors that will provide the full spectrum of wavelengths required. The new power supplies will provide better stability and control of the light beam. And, finally, the new amplifiers and signal control units provide a wider dynamic range and faster operation of the unit than were possible in the prototype system.

Significance to Biomedical Research and the Program of the Institute: This project should provide the specific instrumentation and techniques for the measurement and analyses of the transduction steps which couple the stimulus to the electrical changes in the excitable membrane of photoreceptors. It will also provide a useful tool for similar study of other molecular systems functioning within living cells, for which an increasing need has developed.

Proposed Course of the Project: Once the final instrument is completed, it will be tested out by using objects of known spectral composition in order to determine the optimum operating parameters of the system. Subsequently, we will begin the investigation of visual photoreceptors and some vital staining of intracellular components in photoreceptors and other cells.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-73 LNP/SP-2019
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electrophysiology of Simple Cellular Systems

Previous Serial Number: None

Principal Investigator: Thomas G. Smith, Jr., M.D.

Other Investigators:
Jun Fukuda, M.D.
Phillip Nelson, M.D., Ph.D.[1]
Harold Gainer, Ph.D.[1]
Jeffery Barker, M.D.[1]
Gerald Fischback, M.D.[2]

Cooperating Units:
[1]Behavioral Biology Branch, NICHD
[2]Department of Pharmacology, Harvard Medical School

Man Years:

Total: 2.8
Professional: 2.7
Other: 0.1

Project Description:

Objective: The objective of this research is to gain insight into the membrane mechanisms which are responsible for the excitable responses of single cells.

Methods Employed: The electrical properties of single cells are recorded with intracellular microelectrodes and analyzed under a variety of experimental conditions. The technique of voltage-clamping the membrane potential of single cells is the main method employed in these experiments. This technique requires the penetration of single cells with two microelectrodes, one for monitoring the membrane potential, the other for supplying the current necessary to clamp the membrane potential at the desired level. Using this technique the role of the various membrane ionic conductances responsible for the electrical responses can be studied.

Because of the necessity of taking many measurements from each experiment, the data is recorded on magnetic tape and analyzed with a PDP-12 computer.

Major Findings: This project is the outgrowth of two pilot studies begun during the last fiscal year. The first study involved an investigation of the electrical characteristics of chick muscle cells grown in tissue culture. The second was an analysis of the electrical properties of an identified, neurosecretory pacemaker cell in the ganglion of a snail.

A number of electrical properties of the chick muscle cells have been identified and traced during the course of development of these cells from their immature, myoblastic stage to an apparently "mature" stage. One particularly interesting electric response in these cells is a slow "spike" associated with a slow contraction of the muscle. This slow spike appears quite early i development and disappears with maturation. Preliminary studies suggest that the slow response may be a calcium-potassium spike similar to that found in invertebrate muscle by Prof. Hagiwara and his colleagues; however, the time constants of the conductanc changes in the developing chick muscle are at least an order of magnitude larger than those in invertebrate muscle.

The aim of the pilot study of the snail ganglion cell was to see if the membrane mechanism underlying the bursting, pacemaker activity of the identified cell could be discovered. The voltage clamp analysis suggests that a slow, but regenerative calcium-potassium process may be essential for the pacemaker activity in this neurosecretory cell. In contrast to muscle cells, however, the net ionic driving forces of the pacemaker cell are such that the zero-current or "resting" potential condition does not lie on the steady-steate current-voltage curve but above the negative-slope region of that curve. Hence, the cell has no "resting" potential but oscillates between the two positive-slope regions of the current-voltage curve.

Significance to Biomedical Research and the Program of the Institute: Since this project is new and the experiment results only preliminary, it is premature to be precise or detailed about the actual significance of this research. One can point out, however, a number of areas of its potential significance.

In the first place, the study, in time, of the development o the chick muscle cell in tissue culture may provide insight into the manner in which growth and maturation of excitable membrane process occur and the mechanisms which control these processes. Such insights would be of scientific interest in their own right, but are of potential medical interest for a rational understanding of muscular disorders which may have a developmental basis.

Second, rhythmic or pacemaker activity is a property found in a variety of excitable tissues (nerve cells, heart muscle, and smooth muscle) and any insights concerning the membrane mechanism underlying this activity would be of interest to a number of scientific and medical disciplines.

The third and perhaps most interesting area of potential significance of this research stems from the apparent similarity of the membrane mechanisms underlying two quite distinct physiological processes in completely different tissues, viz. 1) slow contractions in immature chick muscles and 2) periodic oscillations in the membrane potential of a snail cell that controls the release of hormones. Electrophysiologists have heretofore concentrated mainly on the "fast" processes of excitable tissues and have found a considerable ubiquity in nature in the membrane mechanism which underlies these "fast" processes. Many excitable tissues have, however, a variety of "slow" processes which appear to be important in the regulation of physiological activity. It would, therefore, be of considerable interest to know if there were also a ubiquity in nature in the membrane mechanisms underlying these "slow" processes.

Proposed Course of the Project: It is proposed to continue the research on both chick muscle in tissue culture and on the snail cells by means of the voltage-clamp technique. One aim will be a more detailed documentation of the time course of development of the excitable membrane of the chick muscle. In addition, a more complete analysis of the time and voltage dependent ionic conductances underlying the "slow" processes in both the chick muscle and the snail neurons will be attempted. Finally, other tissues which have "slow" processes and are suitable for study will be sought in an effort to find out if they have membrane properties similar to or distinct from those reported here.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-69 LNP/CB-1659
1. Laboratory of Neurophysiology
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Synaptic Contacts of Visual Cells

Previous Serial Number: Same

Principal Investigator: Arnaldo Lasansky, M.D.

Other Investigator: Pier Lorenzo Marchiafava, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 2.6 |
| Professional: | 1.6 |
| Other: | 1.0 |

Project Description:

Objectives: To investigate the fine structure and function of receptors and synapses in the retina.

Methods Employed: Electron microscopy combined with silver impregnations by the method of Golgi. Electrical recordings with intracellular microelectrodes.

Major Findings: During this period the study on the structural organization of the outer plexiform layer of the salamander retina was continued. It was found that rod cell pedicles make ribbon junctions on cone cell processes, and rod cell processes invaginate cone pedicles without otherwise making any specialized contact with them. Horizontal cells make ribbon and distal junctions with the photoreceptor cell pedicles; a single horizontal cell may contact both rods and cones. Bipolar cells were observed to make either ribbon or basal junctions with the photoreceptor cell pedicles; in addition, certain processes believed to belong to bipolar cells make both ribbon and basal junctions with the same or different pedicles. A single bipolar cell may make contact with both rods and cones. Horizontal cells synapse on bipolar cell dendrites and on certain unidentified processes which in turn are also presynaptic to bipolar cells. Ascending branches of these processes invaginate deeply the rod and cone pedicles without otherwise engaging them in any junction. Horizontal cell processes are linked by two kinds of junctions:

close membrane appositions, and contacts analogous to the distal junctions between horizontal cells and rod pedicles.

Electrophysiological studies of the salamander retina have been started, and the light responses of both rod and cone cells have been recorded intracellularly. Both photoreceptors are hyperpolarized by light as reported in other retinas. Rod cells have a lower threshold than cones (about 1 logarithmic unit), a longer time to peak at low light intensities, and a much longer t for recovery after stimulation with bright lights. At this time, the conductance changes associated with the light responses are being investigated.

Significance to Biomedical Research and the Program of the Institute: It is hoped that these observations will help in identifying the mechanisms of synaptic transmission between photoreceptor cells and second order neurons, and provide a bette knowledge of the neuronal networks involved in the processing of visual information within the retina.

Proposed Course of the Project: The light-evoked conductanc changes in rods and cones will continue to be studied in further detail. The possible existence of synaptic interactions between receptors will be investigated by observing the effect on the size of the area covered by the light stimulus, and analyzing the spectral sensitivities of rods and cones.

Honors and Awards: Superior Service Award of DHEW, 1972.

Publications:

Lasansky, A.: Organization of the outer synaptic layer in the retina of the larval salamander, Phil. Trans. Roy. Soc., B., 1973 (in press).

Serial No.:NDS(I)-73 LNP/CB-2016
1. Laboratory of Neurophysiology
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Synaptic Organization of the Inner Plexiform Layer of the Larval Salamander Retina

Previous Serial Number: None

Principal Investigator: Margaret T.T. Wong-Riley, Ph.D.

Other Investigator: William K. Stell, Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1 |
| Professional: | 1 |
| Other: | 0 |

Project Description:

Objectives: The purpose of this study was to investigate the synaptic morphology and organization of the inner plexiform layer of the salamander retina. New criteria for distinguishing between bipolar and amacrine synapses were introduced to replace the less reliable criterion based on the presence or absence of ribbons. The features of the synapses between bipolar, amacrine and ganglion cells were also explored.

Methods Employed: Posterior eye cups of larval salamander were excised and fixed for either light (Golgi-impregnation) or electron microscopic examination. Sets of 100 to 180 serial sections were taken from different animals, and individual processes were traced, whenever possible, to their cells of origin. From the serial sections the consistent morphological characteristics of the bipolar and amacrine processes and ganglion cells dendrites were determined, and the synaptic interconnections between the three types of cells were studied.

Major Findings: New criteria based on the differential morphology of synaptic vesicles and junctional membrane were found, which permit identification of amacrine and bipolar processes in single sections. Amacrine processes contain a fairly uniform population of small round vesicles and they always make conventional synapses of the symmetrical type (that is, both the pre- and post-synaptic membranes have equal thickness). Bipolar processes contain vesicles that are generally larger and more pleomorphic than those of the amacrine processes, and they make ribbon synaps as well as conventional synapses of the asymmetrical type (that is, the postsynaptic membrane is much thicker than the presynapt membrane). Amacrine processes form synapses with other amacrine processes, and also with bipolar and ganglion cells. Amacrine cells also give rise to somatodendritic synapses. Bipolar processes are presynaptic on amacrine processes, ganglion cell dendrites as well as other bipolar processes, but never on the somata of any cell. Both amacrine and bipolar processes are engaged in serial synapses, and these two groups often make reciprocal synapses with each other.

Significance to Biomedical Research and the Program of the Institute: Neuronal processes are more accurately identified through serial sectioning and through the use of aldehyde fixation. The latter method preserves vesicle morphology and junctional membrane characteristics that are distinctive of different types of processes.

The synaptic interconnections between bipolar, amacrine and ganglion cells found in this study provide the morphological bas for future electrophysiological studies in the inner plexiform layer of the retina.

Proposed Course of the Project: A manuscript on the above project is in preparation. The physiological response characteritics of amacrine and ganglion cells to light stimulus will be studied through intracellular recordings and dye injection.

Honors and Awards: None

Publications:

Stell, W.K. and Witkovsky, P.: Retinal structure in the smooth dogfish, *Mustelus canis*: General description and light microscopy of giant ganglion cells. *J. Comp. Neurol.* 148: 1, 1973.

Stell, W.K. and Witkovsky, P.: Retinal structure in the smooth dogfish, *Mustelus canis*: Light microscopy of photoreceptor and horizontal cells. *J. Comp. Neurol.* 148: 33, 1973.

Witkovsky, P. and Stell, W.K.: Retinal structure in the smooth dogfish, *Mustelus canis*: Light microscopy of bipolar cells. *J. Comp. Neurol.* 148: 47, 1973.

Serial No.:NDS(I)-71 LNP/GP-1889
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Agents Affecting Cell Proliferation and Differentiation in the Crystalline Lens

Previous Serial Number: Same

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia A. Grimes, B.A.

Other Investigator: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.8 |
| Professional: | 0.9 |
| Other: | 0.9 |

Project Description:

Objectives: We have observed that a single dose of either locally or systemically administered isoproterenol (IPR) suppresses DNA synthesis and subsequently mitosis in the lens epithelium of rats. In this system IPR acts by stimulating beta adrenergic receptors and cyclic AMP may function as a mediator in effecting the response. The investigation has been extended to the study of the effects of IPR and cyclic AMP and its derivatives on the lens maintained in organ culture to determine whether these agents act directly on the lens to modify cell division.

Methods Employed: Lenses, carefully isolated from young rats were maintained *in vitro* in small petri dishes containing 4 ml of medium 199 supplemented with additional bicarbonate buffer and calcium chloride. The lenses were incubated at 37 deg. C. in equilibrium with a gas phase of 95% air and 5% carbon dioxide. After appropriate intervals the lenses were examined for clarity and fixed; mitotic activity and the number of cells synthesizing DNA were determined by methods previously described.

Major Findings: Cell division and DNA synthesis have not previously been studied in the epithelium of cultured rat lenses.

During the first 24 hours of culture under control conditions, the rate of cell proliferation gradually declines. The number of cells incorporating tritiated thymidine is not significantly different from that in the living animal until 16 hours of isolation, but by 24 hours has decreased to 62% of normal value. Mitotic activity, however, falls steeply in the first two hours after transfer to culture, resumes again by 4 hours, and remains at about 60% of *in vivo* values until 16 hours, after which there is a further fall to 40% at 24 hours. Rat lenses maintained in culture for longer than 24 hours show the general pattern of gross disturbance of cell proliferation and disorganization of the epithelium observed by others in the cultured rabbit lens.

Although it is apparent that the regulation of cell division is altered in cultured lenses, we examined the effects of isoproterenol and other agents during the first 24 hours of isolation. In all cases comparison was made between lenses of the same animal, one placed in control medium and the other in medium containing the drug under test, and the results are expressed in percent of values for the control cultured lenses.

Mitotic activity in lenses cultured in the presence of IPR for 24 hours was slightly depressed at drug concentrations of $1 \times 10^{-4}$, $5 \times 10^{-5}$, and $2.5 \times 10^{-5}$M. No effect was observed at lower concentrations. The concentration of IPR necessary to elicit the suppression was high and the responses were small. In addition there was no fall in the number of DNA synthesizing cells preceding the decrease in mitotic activity such as was seen *in vivo*. It is concluded that under condition of culture, isoproterenol does not reduce the number of cells passing through the proliferative cycle. The fall in mitosis seen at 24 hours may be due to a cumulative, non-specific or possibly toxic action. Further, we have not been able to detect any potentiation of IPR-induced mitotic suppression when lenses are incubated with both IPR and theophylline although such potentiation is clearly evident *in vivo*.

Addition of cyclic AMP ($1 \times 10^{-3}$M) to the culture medium had no significant effect on mitotic activity in the lens epithelium, but both dibutyryl and monobutyryl cyclic AMP were active in suppressing mitosis. This effect, however is clearly different in mechanism from the IPR-induced mitotic suppression *in vivo*. Both mono- and dibutyryl cyclic AMP block mitosis during the $B_2$ period, following completion of DNA synthesis and prior to mitotic division. Inhibition of mitosis is evident after only two hours of culture in the presence of $1 \times 10^{-3}$M dibutyryl cyclic AMP and is maximal at 4 hours when the number of mitotic figures falls to 29% of control counts. Despite the continued presence of the cyclic nucleotide in the medium the number of cells entering mitosis returns to normal by 8 hours.

If dibutyryl cyclic AMP is added to the culture after 20 hours of incubation in control medium, mitosis still falls to 30% of control counts 4 hours later, indicating that the lens does not lose its ability to respond to the cyclic nucleotide during a prolonged period of isolation.

Dibutyryl and monobutyryl cyclic AMP are equally and maximally effective at a concentration of 1 x $10^{-3}$M. The extent of mitotic inhibition at four hours decreases with decreasing concentration of dibutyryl cyclic AMP until at 1 x $10^{-6}$M no further effect is seen. Butyric acid, a possible contaminant and breakdown product of dibutyryl cyclic AMP does not affect mitotic activity in the lens epithelium at concentrations of $10^{-3}$ and $10^{-4}$M.

Significance to Bio-medical Research and the Program of the Institute: The clarification of the effects of these agents on the non-vascularized, non-innervated lens epithelium may contribute to the understanding of the complex mechanisms governing the control of proliferation and differentiation in cell populations of different functional properties.

Proposed course of the Project: Studies will be continued to search for possible intermediary agents involved in the IPR-induced suppression of mitosis such as prostaglandins, and if possible, to provide a link between the different inhibitory phenomena observed *in vivo* and *in vitro*.

Honors and Awards: None.

Publications:

Grimes, P., and von Sallmann, L.: Possible cyclic adenosine monophospate mediation in isoproterenol-induced suppression of cell division in rat lens epithelium. *Invest. Ophth.* 11:231-235, 1972.

Serial No.:NDS(I)-71 LNP/GP-1890
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Organization of a Molluscan Visual System

Previous Serial Number: Same

| | |
|---|---|
| Principal Investigators: | Daniel L. Alkon, M.D.<br>M.G.F. Fuortes, M.D.<br>Peter Detwiler, Ph.D. |
| Other Investigator: | Leona M. Stewart |
| Cooperating Units: | None |

Man Years:

| | |
|---|---|
| Total: | 2.0 |
| Professional: | 1.6 |
| Other: | 0.4 |

Project Description:

Objectives: The principal objective is to understand how information provided by light is processed in the primitive visual system of *Hermissenda crassicornis*, or nudibranch mollusc.

Methods Employed: Responses were recorded intracellularly from receptor and optic ganglion cells. Iontophoretic injection of a fluorescent dye, Procion yellow, was used to study the anatomy of the system.

Major Findings: Further analysis of the second-order cell responses to light as well as the information exchange between the right and left eyes was performed. It was seen that the correlation of the optic ganglion cell response with light intensity is poor. Dim to moderate lights are distinguishable from bright lights, but there is no information concerning gradation of light intensity from dim to bright. The bright flashes do cause a progressively prolonged hyperpolarization related to light intensity. It was suggested that two competing coding processes might be present in the optic ganglion cell response to light: one involving an increase in optic ganglion cell firing (after the flash), the other involving prolonged absence of optic ganglion cell firing (for bright lights).

If movement detection is possible in each eye, as suggested by the responses of photoreceptors, a more specific organization of primary and secondary visual cells seems necessary to preserv this information. The extent of convergence of Type B photoreceptor impingement onto optic ganglion cells, as well as the poor correlation of the optic ganglion cell response with light intensity, suggests, therefore, the existence of second-order visual cells outside the optic ganglion.

Position and movement perception would be facilitated by comparison of visual input to the two eyes whose longitudinal axe are oriented at approximately 90° to each other. Provision for such comparison became apparent in our study of the organization of this molluscan visual system.

Significance to Biomedical Research and the Program of the Institute: The visual system of Hermissenda crassicornis may be the first preparation where careful definition of a neural network is possible. Such definition might contribute to our understanding of visual processing as well as of neural organization in general.

Proposed Course of the Project: This project will be terminated.

Honors and Awards: None

Publications:

Alkon, D.L. and Fuortes, M.G.F.: Responses of photorecepto in Hermissenda. J. Gen. Physiol. 60: 631, 1972.

Alkon, D.L.: Neural organization of a molluscan visual system. J. Gen. Physiol. 1973 (in press).

Fioravanti, R. and Fuortes, M.G.F.: Analysis of responses in visual cells of the Leech. J. Physiol. 227: 173, 1972

Serial No.:NDS(I)-72 LNP/GP-1943
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Spontaneous Retinal Degeneration in Osborne-Mendel Rats

Previous Serial Number: Same

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia A. Grimes, B.A.

Other Investigator: Peter Gouras, M.D.

Cooperating Units: Section on Physiology, LVR, NEI
NEI(I)-73 LVR-005

Man Years:

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.1 |
| Other: | 0.1 |

Project Description:

Objectives: To study the development of a newly-discovered retinal degeneration in mature Osborne-Mendel rats, and to compare this progressive retinal destruction with various forms of human and animal retinal degenerations.

Methods Employed: Rats of various ages from both the inbred and non-inbred colonies of Osborne-Mendel rats maintained at NIH were subjected to repeated electroretinographic testing over a six-month period. The eyes of animals surviving to the end of this interval were examined histologically to determine the extent of photoreceptor degeneration. The electroretinographic and histologic findings were compared to establish appropriate criteria to identify affected animals on the basis of the electroretinogram (ERG).

Major Findings: The retinas of all animals examined up to the age of six month appeared normal on histologic examination. The maximum amplitude of the b-wave of the ERG's recorded from the age of one to six months averaged between 600 and 650 microvolts.

Ten of twelve rats killed between one and two years of age demonstrated retinal degeneration with varying extent of photoreceptor loss. The most typical lesion consisted of partial or total loss of receptor cells in the central retina with a broad band of peripheral retina remaining structurally normal. The maximum amplitude of the b-wave recorded from such affected animals during the six-months preceding death was approximately 20% lower than that recorded from the young apparently normal rats. In general, the average b-wave amplitude for a given animal correlated with the extent of the retinal lesion. Howeve the actual differences in b-wave amplitude between normal animal and those with central retinal degeneration was small, suggesting that the peripheral retina was functionally normal and contr buted in large part to the ERG.

Significance to Bio-Medical Research and the Program of the Institute: The retinal degeneration observed in Osborne-Mendel rats resembles certain human retinal degenerations more closely than do other types of animal retinal dystrophies. It may provide a valuable model for identification of the cause of photoreceptor degeneration in such conditions.

Proposed Course of the Project: ERG recording has been started on a new sample of male and female progeny from each breeding pair within the inbred Osborne-Mendel colony in order to obtain a more inclusive sample of the population and to extend the testing period to detect the onset of photoreceptor loss. We will also examine another inbred colony of rats maintained at NIH (SHR/N), as a similar form of retinal degeneratio has been observed in other colonies of this strain.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-72 LNP/GP-1944
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Organization of a Molluscan Central Nervous System

Previous Serial Number: Same

Principal Investigator: Daniel L. Alkon, M.D.

Other Investigator: Anthony Bak

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.3 |
| Professional: | 1.3 |
| Other: | 0.0 |

Project Description:

Objectives: The principal objective is to understand synaptic mechanisms underlying the interaction of sensory pathways in the primitive nervous system of Hermissenda crassicornis, a nudibranch mollusc.

Methods Employed: Intracellular recording from single hair cells, photoreceptors and visual second-order cells of Hermissenda crassicornis has been used.

Stimulation of the hair cells is accomplished with an electrostrictive device which causes controlled and reproducible displacement. The displacement produces hair cell responses without appreciably disturbing the intracellular electrode.

Major Findings: It was seen that hair cells receive synaptic input from the visual pathway and that photoreceptors are influenced synaptically by hair cell activity. The synaptic organization underlying this interaction appears rather specific. There is little convergence for any one type of ipsilateral visual synaptic input to hair cells although a given hair cell may be affected at the same time by different types of synaptic input such as a Type A and Type B photoreceptor, an ipsilateral optic ganglion cell and a contralateral photoreceptor.

Two principal hair cell light responses were found:

(1) hyperpolarization and cessation of firing
(2) hyperpolarization followed by depolarization and increased firing.

Using a small spot of light to illuminate each eye separately it could be demonstrated that the depolarizing visual input to hair cells arises largely from the ipsilateral eye. The hyperpolarizing input arises from both eyes, but more frequently from the contralateral eye.

It was observed that depolarization may bring out the hair cell response to light. The state of depolarization of a penetrated cell could of course vary with the injury associated with impalement. Since, however, the hair cell also depolarizes with movements of the statocyst, and presumably with changes in orientation, hair cell activity will be more significantly altered when the visual stimulus is associated in time with the appropriate gravitational stimulus. *Hermissenda* was observed to seek light whether in a floating or crawling position. Those hair cells most depolarized for a given orientation of the animal would be expected to be most affected by light. Certain motor neurons might be directly or indirectly influenced by hair cell activity for the animal to move toward light when it is floating and other motor neurons when it crawls. For *Hermissenda*, then, the dependence of a final movement command upon a temporal association of specific visual and gravitation stimuli might account for the positional independence of the animal's phototaxis.

Significance to Bio-medical Research and the Program of the Institute: The visual system and statocyst system (analogous to mammalian vestibular systems) may be the first preparation where definition of intersensory information exchange is possibl Such definition might contribute to our understanding of the visual and vestibular processes and their interactions as well as to neural organization in general.

Proposed Course of the Project: Future research includes the use of a "tilt table" which will allow changes in orientation of the statocyst while recording intracellularly from hair cells and visual cells. Behavioral experiments are also planned to examine the physiologic implications of the interaction of the statocyst with the visual pathway. Pilot experiments indicate it may be possible to correlate electrophysiologi changes in the hair cells' responses to light with changes in behavior.

Finally, experiments are planned to identify more central neurons (possibly motor neurons) which receive input from the statocysts and visual pathways.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-72 LNP/GP-1945
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Neural Organization of the Vertebrate Retina

Previous Serial Number: Same

Principal Investigator: Elliott J. Simon, M.D.

Other Investigators: M.G.F. Fuortes, M.D.
E.A. Schwartz, M.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.6 |
| Professional: | 1.6 |
| Other: | 0.0 |

Project Description:

Objectives: This project is concerned with the elucidation of mechanisms by which electrical responses to light are processed and modified in the vertebrate retina.

Methods Employed: The retina of the turtle has been chosen as the experimental preparation because the anatomy of its synaptic connections has been carefully described by other investigators and because its cells are large enough to permit intracellular recording with microelectrodes. Neurons (primarily photoreceptors and horizontal cells) are impaled with microelectrodes and their responses to different light stimuli are determined. Identification of the cells from which electrical responses originate is determined by intracellular marking by dye iontophoresis.

Major Findings: Cone photoreceptors in the turtle retina contain visual pigments which maximally absorb either red, green or blue light. Absorption of light by a cone elicits in it a slow hyperpolarizing response which is graded with light intensity ("direct response"). Stimuli of any color which cover small areas will produce the same direct response if their intensities are adjusted appropriately.

Luminosity horizontal cells ("L-cells") are syanptically connected to the photoreceptors and to other L-cells. Experiments performed in this study have shown that L-cells in the turtle respond to large areas of retinal illumination due to electrical coupling between them. Two types of L-cell were identified which have different receptive fields and different morphology as determined by intracellular dye marking.

One type of L-cell feeds a depolarizing wave back onto cone when the illuminated field is large (Baylor, Fuortes, and O'Brya J. Physiol., 1971); this wave combines with the direct response to give a composite cone response. In this study we have shown that the input to these L-cells includes cones of different colors; consequently, the feedback component of the composite cone response has a mixed color input. Because of this organization the net cone response differs for different colors of stimulation even when the direct responses are identical. Depending on the color, intensity, and pattern of illumination the cone response varies in size, shape, or polarity.

Other studies have shown that the responses of rod photoreceptors are also modified by illumination of nearby receptors. In addition, it has been demonstrated that cells which produce spikes at the on and the off of a light may be either amacrine or ganglion cells; the activity of these cells is antagonized by peripheral illumination, and this antagonism is not mediated by the horizontal cells.

Significance to Biomedical Research and the Program of the Institute: The retina is one of few parts of the vertebrate central nervous system which is simple and accessible enough to permit the detailed cellular investigations necessary to determine fundamental principles of organization of the CNS. The input (light stimuli) and the output (optic nerve activity) of the retina can be fully determined. All processing which transforms the light into the complex neural codes of the output is performed by only five cell types all of which can be studied by intracellular recording. This project is designed to elucidae the mechanisms by which the various retinal cells interact to process visual information.

Proposed Course of the Project: Current and future investia tions include further study of the interactions of first order (receptors) and second order (horizontal cells and bipolar cells neurons which will permit a detailed description of the electricl circuitry of the outer part of the retina. In particular, we shall investigate how the responses of "chromatic horizontal cels which have electrical behavior determined by the color of a visual stimulus are produced, and how their responses combine wit those of L-cells and receptors to generate responses in bipolar cells, which are the only link to third order retinal neurons.

Honors and Awards: None

Publications:

Fuortes, M.G.F.: Responses of cones and horizontal cells in the retina of the turtle. Investigative Ophthalmology. 11: 275, 1972.

Fuortes, M.G.F. and O'Bryan, Paul M.: Generator Potentials in Invertebrate Photoreceptors (Chapters 6 and 7). In Handbook of Sensory Physiology, Vol. VII/2. Berlin-Heidelberg-New York, Springer-Verlag, 1972.

Schwartz, E.A.: Organization of On-Off cells in the retina of the turtle. J. Physiol. 1973 (in press).

Schwartz, E.A.: Responses of single rods in the retina of the turtle. J. Physiol. 1973 (in press).

Simon, E.J.: Two types of luminosity horizontal cells in the retina of the turtle. J. Physiol. 1973 (in press).

Serial No.:NDS(I)-73 LNP/GP2017
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mechano Receptors: Organization and Function

Previous Serial Number: None

Principal Investigators: Peter Detwiler, Ph.D.
Daniel L. Alkon, M.D.

Other Investigator: Anthony Bak

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 2.5 |
| Professional: | 2.5 |
| Other: | 0.0 |

Project Description:

Objectives: To study the process of mechano-reception in the invertebrate hair cell. Our study may be divided into two parts. First, how is a mechanical stimulus, e.g. membrane deformation, transduced into a membrane potential change. Second, how do hair cells interact with each other.

Methods Employed: Intracellular recordings were obtained from single or pairs of hair cells in the statocyst (a primative vestibular apparatus) of the invertebrate *Hermissenda crassicornis*. Hair cells were mechanically stimulated by a piezoelectric device.

Major Findings: Hair cells respond to a mechanical stimulus with a depolarization that is frequently followed by a marked hyperpolarization and always followed by an inhibition of spike activity. The depolarization is a direct result of mechano-transduction. It occurs with a short delay (<2ms), is due to a conductance increase for one or more ions, does not adapt to low frequency stimulation (<10cps) and is temperature sensitive. The mechanically-evoked hyperpolarization and spike inhibition are a synaptic phenomenon due to inhibitory interactions between hair cells. Hair cells may interact synaptically with each other either electrically or chemically. All chemical interactions are inhibitory and may be either unidirectional or reciprocal. Hair cell pairs that showed inhibition in one direction were generally located 90° to each other on the statocyst. Hair cell pairs that showed reciprocal inhibition were always located 180° to each other.

Significance to Bio-Medical Research and the Program of the Institute: All animals from the unicellular paramecium to man have the ability to respond to mechanical stimulation. In higher organisms mechano-receptors provide information about a greater spectrum of stimuli than any other single receptor. In man, for example, mechano-receptors are responsible for the skin sensations of touch and pressure, for muscle and internal organ proprioception, for joint position sense, for gut reflexer that give rise to peristalsis, for cardiovascular reflexes dependent upon blood pressure, for hearing, and for the senses of balance, acceleration, and direction of gravity. In spite of its widespread distribution and fundamental importance mechano-reception is a poorly understood process. The reason for this is technica: mechano-receptors are generally small and difficult to study. Our work represents only the second in depth intracellular study of a mechano-receptor and the first of a hair cell (hair cells are the receptors that subserve hearing and vestibular sensation in man). This work provides basic information about mechano-reception in general and hair cell function in particular.

Proposed Course of the Project: During the next year we plan to extend our previous studies and begin working on the relationships between hair cells and motor cells. In this manner we hope to have a better understanding of the entire mechano-transduction process from its initiation with mechano-transduction to its motor output.

Honors and Awards: None

Publications:

Alkon, D.L., and Bak, A.F.: Hair cell generator potentials J. Gen. Physiol. 1973 (in press).

Serial No.:NDS(I)-73 LNP/GP-2018
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Ionic and Pharmacologic Basis of the Inhibitory Interaction Between Photoreceptors of a Simple Visual System

Previous Serial Number: None

Principal Investigator: Leona M. Masukawa

Other Investigator: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.5 |
| Professional: | 0.5 |
| Other: | 0.0 |

Project Description:

Objectives: The objective of this project is to understand the ionic and pharmacologic mechanisms operating in a synaptic system of a very simple visual organ.

Methods Employed: Experiments were performed using the visual organ of Hermissenda crassicornis. The visual organ is a simple eye composed of five photoreceptor cells which are all interconnected by inhibitory synapses. These were the synapses studied for the above objective. Intracellular recording methods were used combined with a constant perfusion technique to alter the ionic and drug environment of the preparation.

Major Findings: The inhibitory synapses were found to be dependent on the potassium ion concentration and very little on chloride ion concentration.

The synapses are not blocked by the following drugs in $10^{-4}$M concentration: atropine, d-tubocurarine, picrotoxin, and tryptamine. The photoreceptor cells are not affected by $10^{-4}$M concentrations of dopamine, or 5-hydroxytryptamine.

The synapses were however found to be blocked by calcium ion antagonists, (cobalt and magnesium) in the classical ways as shown at chemical synapses.

Significance to Biomedical Research and the Program of the Institute: Because of the simplicity and accessibility of the visual system and its synapses, research can be carried out which can aid in the advancement of research in more complex and technically inaccessible visual systems and synapses.

Proposed Course of the Project: Further work into the influence of sodium ion concentration and the possible action of electrogenic pumps on the inhibitory synapse will be carried out. Also the pharmacologic study will be completed in an attempt to identify possible transmitter candidates.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-71 LNP/NI-1892
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Comparative Physiology of Invertebrate Photoreceptors

Previous Serial Number: Same

Principal Investigator: John S. McReynolds, M.D.

Other Investigator: Anthony L.F. Gorman, Ph.D.

Cooperating Units: Department of Neurobiology,
Marine Biological Laboratory
Woods Hole, Massachusetts

Man Years:

| | |
|---|---|
| Total: | 1.2 |
| Professional: | 1.2 |
| Other: | 0.0 |

Project Description:

Objectives: The overall objectives of this project are to study in different ways the mechanisms by which sensory receptor neurons convert external stimulus energy into the electrical signals which are transmitted to higher centers in the nervous system. In particular, the present work is concerned with the control of membrane permeability and membrane potential of the primary sensory neuron by the stimulus.

Methods Employed: Intracellular recording with glass microelectrodes were used to measure the membrane potential of single receptor neurons in the retina of a mollusc, Pecten irradians. Previous work, described in earlier annual reports of this project, has revealed that this preparation has a number of features which make the study of these receptors of special interest. Measurements of membrane resistance under different conditions were also made. Continuing the previously reported work, the effect of specific changes in the extracellular ionic medium were studied. Also, the effect of various metabolic inhibitors on membrane potential and permeability were investigated.

Major Findings: This work has established that the primary electrical response (the receptor potential) in these sensory neurons is due to a selective increase in potassium permeability of the cell membrane, caused by the actions of the stimulus. In all other receptors so far studied, the receptor potential has been found to be due mainly to a change in membrane permeability to sodium ions. On *Pecten*, instead, the receptor potential is produced by a selective increase in permeability to potassium ions. The preliminary studies with metabolic inhibitors indicate that there is also a link between cellular metabolism and the membrane permeability to potassium. The inhibitors 2,4-dinitrophenol and cyanide increase potassium permeability and hyperpolarize the cell. These findings suggest that potassium permeability (and therefore the membrane potential) is maintained at the "resting" level by an active metabolic process.

*Significance to Biomedical Research and the Program of the Institute*: Although a great deal has been learned about the initial photochemical reactions in visual receptors, and about the changes in membrane permeability which generate the sensory neuron's electrical signal, very little is known about the mechanisms which control membrane permeability of sensory receptors. This gap applies to our knowledge of the visual syste The findings that the receptor potential may be generated by a selective increase in potassium permeability, and that membrane permeability may also be regulated by metabolic activity, may be important to our understanding of how sensory neurons function

*Proposed Course of the Project*: Investigation of the possible role of calcium ions in the regulation of membrane $K^+$ permeability.

Honors and Awards: None

Publications:

Gorman, A.L.F., and McReynolds, J.S.: Potassium dependence of the hyperpolarizing receptor potential of the scallop photoreceptor. *Biol. Bull.* 143: 464, 1972.

Serial No.:NDS(I)-71 LNP/NI-1894
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Synaptic Transmission Between Photoreceptors and Horizontal Cells in the Turtle Retina

Previous Serial Number: Same

Principal Investigator: Luigi Cervetto, M.D.

Other Investigator: Edward F. MacNichol, Jr., Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.3 |
| Professional: | 0.3 |
| Other: | 0.0 |

Project Description:

Objectives: The objective of this research is to complete the first step in an orderly progression of investigations of synaptic transmission through the layers of the vertebrate retina. This is the elucidation of the mechanism of information processing in the receptors and of information transfer at the synapses between the receptors and horizontal and bipolar cells.

Methods Employed: Retinae were maintained in the eyecup with solution flowing over the internal limiting membrane while gross ERG responses were recorded with extracellular electrodes and intracellular responses with micropipette electrodes. Thanks to the work of Tomita et. al., Kaneko, Werblin and Dowling, and Baylor and Fuortes it is possible to distinguish which cells have been penetrated by micropipette electrodes by purely electrical criteria so that the responses of receptors, horizontal cells, and bipolar cells can be clearly distinguished from one another.

The action of presumed synaptic transmitters and of agents which block the synapses was investigated. In addition the effects of ions on membrane potentials and on responses was studied.

Major Findings: 1. L-Glutamate and L-Aspartate which suppress the b wave of the ERG was shown to depolarize horizontal cells and to block synaptic transmission between receptor and horizontal cells. The feedback depolarization of the receptor subsequent to the hyperpolarization produced by light was also abolished. Thus the use of glutamate and aspartate to study receptor responses in isolation has been validated. 2. The influence of sodium, potassium and chloride ions upon the response of the photorecept and horizontal cells were studied. From the behavior of the membrane potential in light and darkness when ionic concentratio were changed the following conclusions were reached: a. In darkness the photoreceptor membrane is partially depolarized because the permeability to sodium is higher than the permeabilit of potassium. b. During illumination the permeability to potassium becomes higher than the permeability to sodium: thus the receptor hyperpolarizes. Since Tomita et. al. have shown that the membrane resistance of receptors increases during illumination it can be concluded that the hyperpolarization is due more to a decrease in sodium permeability than to an increase in permeability to potassium.

Significance to Biomedical Research and to the Program of the Institute: The vertebrate retina is both functionally and ontogenetically a portion of the central nervous system. Because of its layered structure, its small thickness which permits ready penetration of applied solutions, and its susceptibility to precisely controlled natural sensory stimuli it is ideally suited to the study of the mechanisms of conduction and transmission in short neurons, and of synaptic organization. The knowledge gaine from the study of retinal synapses which use amino acid transmitters should be applicable to elucidatory synaptic function elsewhere in the CNS.

Proposed Course of Project: Due to the departure of Dr. Cervetto in September 1972 and the imminent departure of Dr. MacNichol before the end of the fiscal year the project is no longer active. The investigators plan to continue the studies independently at the University of Pisa, Italy and at the Marine Biological Laboratory, Woods Hole, Massachusetts.

Honors and Awards: None

Publications:

Cervetto, L. and MacNichol, E.F., Jr.: Inactivation of horizontal cells in the turtle retina by glutamate and aspartate. Science. 178: 767, 1972.

Cervetto, L.: Influence of sodium, potassium and chloride ion on the intracellular responses of turtle photoreceptors. Nature. 241: 401, 1973.

Serial No.:NDS(I)-71 LNP/NI-1895
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Microspectrophotometric Studies of Vertebrate Photoreceptors

Previous Serial Number: Same

Principal Investigator: Ferenc I. Harosi, Ph.D.

Other Investigator: Edward F. MacNichol, Jr., Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.6 |
| Professional: | 1.5 |
| Other: | 0.1 |

Project Description:

Objectives: This project is aimed at the identification of the absorptive properties of vertebrate visual cells.

Methods Employed: Freshly isolated rod and cone cells obtained from dark-adapted retinas are suspended in solutions of various composition. Thin preparations of such suspensions are used to measure single cells in a microspectrophotometer. Absorption spectra are recorded and linear dichroism is measured in the dark and following brief flashes of bright light. The formation and decay of photoproducts are detected in terms of spectral properties and dichroism at room temperature and under selected ionic conditions.

Major Findings: A. Rhodopsin-containing frog rods: 1) During the first second following a white flash there is a rapid spectral transformation of the visual pigment from $\lambda_{max}$=502nm to $\lambda_{max}$=380nm. The average orientation of the chromophore initially remains transverse (that is the dichroic ratio of 4-5:1 remains unchanged from rhodopsin to metarhodopsin II), but then turns axial (that is the dichroic ratio falls and finally reverses). Since this dichroic transition takes several minutes, it is probably associated with major protein conformational changes. The initial spectral transition, on the other hand, does not appear to be the result of large conformational changes. 2) The final product of bleaching in freshly isolated cells is probably vitamin $A_1$; its average chromophore orientation is predominantly axial. This substance is not produced in old cells (that is, in cells which are bleached several hours following their removal from the retina). 3) The lack of calcium (and presence of EGTA in the suspending medium appears to slow down the decay of intermediates. 4) The specific optical density (that is, the cell's optical density divided by its diameter) for transversely polarized light is 0.017±0.0015/μm.

B. Goldfish cones: 1) We confirm the finding of three types of cones in the goldfish retina, each containing a spectrally pure visual pigment. Their absorption maxima and estimated error margins are: 455±3, 530±3 and 625±5 nm. 2) Sid illuminated cone outer segments are dichroic. The apparent preference for absorbing transversely polarized light (Measured dichroic ratio) is 2-3:1. 3) We confirm and extend earlier observations concerning correlations between visual pigment content and gross cone morphology. 4) The spectral maxima of the detected photoproducts, taken together with other evidence, indicate that the aldehyde of vitamin $A_2$ is used by the cone pigments as chromophore. 5) The rate of decay of photoproducts in goldfish cones is about five times as rapid as that in frog rods (when measured under similar conditions). 6) The average specific optical density of cone outer segments (in side view and for transversely polarized light) is 0.0124±0.0015/μm. 7) The half-band widths of cone pigment spectra are such that the types B and G (Blue and Green) match well the appropriate phorphyropsin standards, having $\Delta\nu = 4832 \pm 100\ \mathrm{cm}^{-1}$. 8) The type R (Red) spectrum is significantly narrower than the standard and has $\Delta\nu = 3625 \pm 100\ \mathrm{cm}^{-1}$. 9) The specific density and spectral width determinations are consistent with the notion that the visual pigment packing (concentration) is about the same in frog rod and goldfish cone outer segments, but that the types B and G pigments have a molar extinction of only about 30,000 liter/mol. cm. 10) Based on the hypothesis that the oscillator strength of the main band is invariant for the three types of cone spectra, the molar extinction for the type R pigment is calculated to be about 40,000 liter/mol. cm.

C. Turtle cones: We have so far been unable to make retinal preparations from turtles with unfragmented cones and hence to detect visual pigments. Preliminary measurements on the colored oil droplets located in cone ellipsoids indicate that there are at least four spectrally distinct types. Their cut-off wavelengths (at 50% of maximal absorptance) were found at about 415, 535, 560 and 595 nm. The chromophores of these pigment molecules show random orientation (no dichroism). It also appears likely that these pigments are chemically distinct substances.

Serial No.:NDS(I)-71 LNP/NI-1895
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Microspectrophotometric Studies of Vertebrate Photoreceptors

Previous Serial Number: Same

Principal Investigator: Ferenc I. Harosi, Ph.D.

Other Investigator: Edward F. MacNichol, Jr., Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.6 |
| Professional: | 1.5 |
| Other: | 0.1 |

Project Description:

Objectives: This project is aimed at the identification of the absorptive properties of vertebrate visual cells.

Methods Employed: Freshly isolated rod and cone cells obtained from dark-adapted retinas are suspended in solutions of various composition. Thin preparations of such suspensions are used to measure single cells in a microspectrophotometer. Absorption spectra are recorded and linear dichroism is measured in the dark and following brief flashes of bright light. The formation and decay of photoproducts are detected in terms of spectral properties and dichroism at room temperature and under selected ionic conditions.

Major Findings: A. Rhodopsin-containing frog rods: 1) During the first second following a white flash there is a rapid spectral transformation of the visual pigment from $\lambda_{max}$=502nm to $\lambda_{max}$=380nm. The average orientation of the chromophore initially remains transverse (that is the dichroic ratio of 4-5:1 remains unchanged from rhodopsin to metarhodopsin II), but then turns axial (that is the dichroic ratio falls and finally reverses). Since this dichroic transition takes several minutes, it is probably associated with major protein conformational changes. The initial spectral transition, on the other hand, does not appear to be the result of large conformational changes. 2) The final product of bleaching in freshly isolated cells is probably vitamin $A_1$; its average chromophore orientation is predominantly axial. This substance is not produced in old cells (that is, in cells which are bleached several hours following their removal from the retina). 3) The lack of calcium (and presence of EGTA in the suspending medium appears to slow down the decay of intermediates. 4) The specific optical density (that is, the cell's optical density divided by its diameter) for transversely polarized light is 0.017±0.0015/μm.

B. Goldfish cones: 1) We confirm the finding of three types of cones in the goldfish retina, each containing a spectrally pure visual pigment. Their absorption maxima and estimated error margins are: 455±3, 530±3 and 625±5 nm. 2) Sid illuminated cone outer segments are dichroic. The apparent preference for absorbing transversely polarized light (Measured dichroic ratio) is 2-3:1. 3) We confirm and extend earlier observations concerning correlations between visual pigment content and gross cone morphology. 4) The spectral maxima of the detected photoproducts, taken together with other evidence, indicate that the aldehyde of vitamin $A_2$ is used by the cone pigments as chromophore. 5) The rate of decay of photoproducts in goldfish cones is about five times as rapid as that in frog rods (when measured under similar conditions). 6) The average specific optical density of cone outer segments (in side view and for transversely polarized light) is 0.0124±0.0015/μm. 7) The half-band widths of cone pigment spectra are such that the types B and G (Blue and Green) match well the appropriate phorphyropsin standards, having $\Delta\nu = 4832\pm100$ cm$^{-1}$. 8) The type R (Red) spectrum is significantly narrower than the standard and has $\Delta\nu = 3625\pm100$ cm$^{-1}$. 9) The specific density and spectral width determinations are consistent with the notion that the visual pigment packing (concentration) is about the same in frog rod and goldfish cone outer segments, but that the types B and G pigments have a molar extinction of only about 30,000 liter/mol. cm. 10) Based on the hypothesis that the oscillator strength of the main band is invariant for the three types of cone spectra, the molar extinction for the type R pigment is calculated to be about 40,000 liter/mol. cm.

C. Turtle cones: We have so far been unable to make retinal preparations from turtles with unfragmented cones and hence to detect visual pigments. Preliminary measurements on the colored oil droplets located in cone ellipsoids indicate that there are at least four spectrally distinct types. Their cut-off wavelengths (at 50% of maximal absorptance) were found at about 415, 535, 560 and 595 nm. The chromophores of these pigment molecules show random orientation (no dichroism). It also appears likely that these pigments are chemically distinct substances.

Significance to Biomedical Research and the Program of the Institute: The twofold aim of this project, namely 1) to characterize the abscrptive properties of vertebrate photoreceptors, and 2) to understand the photo-chemical process of bleaching in visual pigments, are important for both the electrophysiological studies of the retina as well as the elucidation of the molecular mechanisms underlying vision in vertebrates including man.

Proposed Course of the Project: 1) to continue the study of turtle cones. 2) to complete the modification of the instrument for temperature-controlled experiments. 3) to study the kinetics of photo-chemical reactions in frog rods and goldfish cones. 4) to measure visual pigments of primate cones.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-71 LNP/NI-1934
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electroencephalograph Calibrator for Use in Determining Electrocerebral Silence

Previous Serial Number: Same

Principal Investigator: Edward F. MacNichol, Jr., Ph.D.

Other Investigator: None

Cooperating Units: Technical Development Section NIMH/NINDS
Electroencephalography Branch
Office of Biometry
Office of Information

Man Years:

| | |
|---|---|
| Total: | Less than 0.1 |
| Professional: | Less than 0.1 |
| Other: | 0 |

Project Description:

Objectives: The development of a method for use in cases of suspected cerebral death.

Methods Employed: An objective method of determining whether or not electrocerebral activity would be recorded if present is badly needed. Furthermore, electrocerebral silence is an important criterion upon which a decision to terminate resuscitative measures is based. Medico legal questions are involved, particularly when the patient is a potential donor of vital organs.

An initial attempt to furnish a test signal that would check the entire system was the development of a constant current square wave generator having a frequency of about 1Hz that would inject a signal between chin and vertex of the patient. A pilot model of this device (Injector) was built by the Technical Development Section and tested on a series of patients in the Electroencephalography Branch. The ratios of output potential in various lead pairs to input current was measured.

Major Findings: Analyses of the records by the Office of Biometry indicate a large variation in output between different leads on the same patient and within identical leads on different patients. Thus the presence of a tracing on a given channel sho that the system is working and that the electrodes are functiona connected to the patient, but does not provide a known calibrati signal to compare with the amplitude of the EEG. A commerical version of this device developed independently by the Grass Inst ment Company, is being made available to investigators for evalu tion.

In order to provide a calibrating signal a closed transform core with a primary winding is used. One of each pair of EEG le is threaded through the aperture in the core and a suitable wav form applied to the primary to induce a 1Hz square wave of known amplitude in each single turn secondary. Since the half period of the 1Hz square wave is much below the L/R time constant of th primary the transformer takes the time derivative of the input waveform, which is therefore a symmetrical ramp function. This is generated by a square wave input which is compared with the output of a feedback winding on the core.

To determine whether the leads are connected to the patient scalp and to provide a known calibrating signal both devices are needed. A combined device has been constructed.

Significance to Biomedical Research and the Program of the Institute: The NINDS Council has urged the Institute to undertake a study of cerebral death. This study is entering its pilot phase in six collaborating institutions. It is hoped that the prototype units can assist this study by providing indications of the functional state of the EEG systems to those who wi evaluate the records.

Proposed Course of the Project: This project has been terminated. The units are still undergoing field evaluation.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-72 LNP/NI-1946
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Statocyst Receptor Physiology

Previous Serial Number: None

Principal Investigator: Michael L. Wiederhold, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.0 |
| Professional: | 1.0 |
| Other: | 0.0 |

Project Description:

Objectives: These experiments were undertaken to study ciliated mechanoreceptors analogous to the hair cells of the mammalian inner ear and vestibular system.

Methods Employed: Invertebrate statocysts are analogous to the semicircular canal system of mammals. The receptor cells bear cilia similar to those of the hair cells of the mammalian inner ear and semicircular canals. Thus, it is hoped that these simple, more accessible receptors will provide a useful model for the functions of the receptors in more advanced animals.

Major Findings: Intracellular recordings have been made from a number of statocyst receptors in Aplysia. The cells respond to tilting of the whole preparation with a depolarization, which, if of sufficient amplitude, generates spike activity. This depolarization is associated with a decreased membrane resistance. The input resistance of these receptors has been observed to decrease by as much as 40% when they are stimulated. The passive current-voltage characteristics of the receptor is also being studied. Most cells in a healthy preparation have an input resistance of from 20 to 40 megohms and time constant of approximately 5 msec.

Significance to Bio-medical Research and the Program of the Institute: Similarities in fine structure suggest that the functions of these receptors are similar to those of the mammalian inner ear and vestibular system. Due to the technica difficulties of studying the latter directly, little informatio is available concerning the functions of mammalian hair cells. The larger and more accessible receptors in *Aplysia* provide th possibility to study directly the changes in the receptor cell membrane brought about by deformation of the sensory cilia which lead to excitation. If the ototoxic drugs such as Kanamycin and Streptomycin also affect this system, the statocyst will also provide the possibility of studying the physiological effect of these drugs.

Proposed Course of the Project: This project has been terminated at NIH due to the transfer of the principal investigator. It is being continued with DOD funds at the Radiobiology Research Institute, NNMC, Bethesda, Maryland.

Honors and Awards: None

Publications:

Wiederhold, M.L., MacNichol, E.F. Jr., and Bell, A.: Photoreceptor responses of the hardshell clam, *Mercenaria, Mercenaria* *J. Gen. Physiol*. 61: 24, 1973.

Annual Report
July 1, 1972 through June 30, 1973
Laboratory of Biophysics, IR
National Institute of Neurological Diseases and Stroke

William J. Adelman, Jr., Ph.D., Chief

## Introduction

The overall research program of the Laboratory of Biophysics has been concerned with uncovering basic molecular mechanisms associated with the generation of the nerve impulse and with the maintenance of resting ionic gradients across neural membranes. This program has involved the study of natural excitable membranes, artificial membrane systems, ionophores extracted from a variety of natural membranes, ionophores of bacterial origin and the theoretical study of membrane excitability and neural function. The Laboratory has made wide use of physical and chemical techniques, on-line and off-line digital computers, and a variety of applied mathematical methods. In the course of these studies, the Laboratory has expanded its cooperation with other laboratories in the NIH as well as with those in universities. Some considerable effort of the Laboratory has been spent in re-tooling so as to expand its base in the study of biophysical mechanisms associated with neuroactivity. The Laboratory's overall contributions to biophysics and general neurophysiology have been recognized by the number and variety of invitations to give major reviews and lectures. Invited review lectures have been given by staff members at the Biophysical Society Annual Meeting in Columbus, Ohio, at the American Association for the Advancement of Science Annual Meeting in Washington, D. C., at the Smithsonian Institute Neurobiology Symposium and at the Marine Biological Laboratory Excitable Membrane Program. Five major review articles have been published in "Perspectives in Membrane Biophysics", in "Current Topics in Membranes and Transport", in "Challenging Biological Problems" and in "Annual Reviews of Biophysics and Bioengineering". On November 30, 1972, Dr. Kenneth S. Cole was admitted into the Royal Society as a Foreign Member. Overall, the Laboratory continues to expand its basic scientific approach into new areas of biophysics, consolidate and strengthen its work of the past decade, and to search for basic mechanisms responsible, in part, for certain clinical disorders, and for the molecular basis for certain drug and toxin actions. Research program of the Laboratory is summarized below:

## ATPase

Three major projects involving the enzyme adenosine triphosphatase, ATPase, were undertaken by the Laboratory. ATPase is universally important in ion transport systems in neural and other tissues. (1) The first project has resulted in the discovery, isolation, characterization of a $Na^+$ - specific ionophore from $Na^+$-$K^+$-ATPase obtained from eel electroplax and beef kidney membranes. This work was done in collaboration with the Laboratory of

Neurochemistry, NINDS. By making use of artificial bilayer techniques now standardized by Laboratory of Biophysics personnel, the Na$^+$-ionophore has been shown to have a voltage dependency similar to that reported for alamethicin. It appears that six units may be required to achieve an active conductive unit. The Na$^+$-ionophore has been shown to have a molecular weight of about 2000. (2) A Ca-ATPase system has been studied in collaboration with the Department of Oral Biology of the University of Connecticut. A decrease in Ca-ATPase was found in Vitamin D deficient rats. It appears that Vitamin D deficiency decreases both Ca-ATPase levels and Ca-absorption in the intestine. However, in the kidney, Ca-ATPase decreases without a parallel decrease in Ca-reabsorption as a result of Vitamin D deficiency. However, in this case, an increase in Na+K-ATPase was observed in the kidney. The increase in Na+K-ATPase in the kidney may account for the normal renal Ca-reabsorption and suggests that there may be Ca-Na coupling in the kidney. (3) In collaboration with the Department of Physiology, George Washington University and the Department of Physics, University of Louisville, a study has been undertaken to examine the role of iron in Na+K-ATPase. Fe$^{++}$ and not Fe$^{+++}$ was shown to specifically inhibit Na+K-ATPase. The Mossbauer spectrum of Fe$^{++}$ was utilized to elucidate the molecular mechanism of Na+K-ATPase. It was found that ouabain increases the quadrupole splitting Mossbauer spectrum of Fe$^{++}$ in the presence of Mg, Na, and K. This finding suggests that ouabain keeps two charge centers separated by a greater distance than the charge separation distance indicated for no ouabain inhibition.

## Channel noise in membranes

Recently, the response of giant axons to white-noise stimuli has been investigated. It appears that this technique has some virtues as a means of measuring membrane impedance and also (with the proper analysis) as a means of obtaining the statistical properties of spike trains in a rapid way. An analysis of the response of the Hodgkin-Huxley model to white noise current stimulation was worked out, at first using the Wiener theory of nonlinear systems. This provided some limited results for the subthreshold domain. A reasonable account of spike train statistics was produced by using a noise theory developed by Stratanovich. In order to observe fluctuations caused by the opening and closing of ion-conducting channels the electrical noise associated with this activity is under investigation. Analysis of the noise detection problem in excitable cells has indicated that the most sensitive system involves the voltage clamp of micron sized membrane patches in which the conductance fluctuations represent a large part of the mean conductance. The best candidates for such a study seem to be tissue cultured nerve cells which have little, if any, basement membrane and no connective tissue or glia. Electrodes have been developed which have a micron or smaller hole in a large diameter tube (5-10 micra) and exhibit resistances comparable to the convergence resistance for such a hole. Work is proceeding in further development of the electrodes to lower noise levels, and preliminary experiments indicate that it is possible to seal them to neuroblastoma cells in culture. Statistical measures of the noise have been developed and are being programmed onto the PDP-11 data acquisition system.

## Intracellular water structure

The studies on the structure of intracellular water using spin labelled single muscle fibers were continued using electron paramagnetic resonance techniques. These studies on water structure have shown that the motion of the spin probe both in muscle fibers and in albumin solutions is not a single process, and probably involves a fast exchange between the freely tumbling radicals and a bound fraction. The fast exchange situation may also occur for other solutes in cells, accounting for low conductivities and activities. A manuscript describing this work has been completed and is in the process of being submitted for publication.

## On-line data acquisition by a digital computer system and subsequent data analysis

In November 1972, a data acquisition system incorporating a disk operating PDP-11 digital computer with a nine-track read write magnetic tape peripheral was obtained by the Laboratory. A sophisticated interface incorporating extremely rapid analog to digital conversion under computer control was constructed in the Laboratory with the aid of the Section on Technical Development, NIMH/NINDS. This interface was designed to acquire data in real time from a variety of experimental preparations of interest to the Laboratory. With the completion of the data acquisition interface for the PDP-11, programs are being developed that are necessary for use of the interface to control experiments and record, display, and analyze data. A display system for the PDP-11's cathode ray tube and plotter is nearly complete. A program has been produced which makes sufficient use of the data acquisition interface to give it a reasonable test. Reduction of data by DCRT's PDP-10 system or by other batch-processing computers is still not completely set up. The PDP-11 is now being programmed to plot various functions and solutions to real problems on DECwriter, and on a graphic recorder. A major effort is being made to achieve rapid and meaningful data anlysis through preliminary PDP-11 processing and subsequent PDP-10 analysis. MLAB (on the PDP-10) works well for certain parameters but extensions to MLAB have to be worked out for economical and efficient use. Simulation worked fine for $R_s$ computations. The small SPC-12 on-line system is being made compatible with the PDP-11 system. Until the PDP-11 system becomes fully operational, the SPC-12 system remains the major Laboratory data acquisiton system. The micro-LINC still can give only minimal data reduction, curves and printouts. The LINC is used to collect data and for program controlled voltage clamping. At present, the voltage levels are not large enough to properly clamp an axon. These levels have changed and are being brought back to what they were before. With the PDP-11, problems encountered with the LINC are no longer a concern.

## Periaxonal potassium accumulation and diphenylhydantoin

Two projects of the Laboratory have been basic to our understanding of some of the processes possibly contributing to epileptiform discharges and their management with drug therapy. The project on $K^+$ accumulation in periaxonal space has led to a general model. In the central nervous system natural repetitive activity of axons and neurons must result in perineuronal

$K^+$ accumulation. The resulting changes in perineuronal $K^+$ concentration will affect the firing rates, integrative functions, transmitter release, etc., in these systems. Using MLAB, modified Hodgkin-Huxley equations incorporating K accumulation have been solved for repetitive firing and predict phenomena observed during spike trains. Phenomena such as spreading cortical depression and epileptiform cortical discharges have been explained on the basis of changes in perineuronal $[K^+]$, modifying the activity of both peripheral and central nervous systems. This consideration has been reviewed and compared with experimental and theoretical work on squid giant axons (Adelman and Palti, 1972).

Internal perfusion of the squid giant axon has been achieved with the anti-epileptic chemical, diphenylhydantoin. The effect of this procedure is to decrease both the sodium and potassium currents. This work has provided us with clues as to the molecular basis for the action of diphenylhydantoin--namely, that as a sodium and potassium channel blocker, the drug raises general neural thresholds, making neural cells less excitable.

## Heavy metal ions

A number of projects have, in part, been concerned with the effects of heavy metal ions on peripheral nerve activity. Lead has been shown to block sodium and potassium channels in *Myxicola* axons. In a cooperative study with the State University of New York at Binghamton and Juniata College, the transition metal ions in the rare earth lanthanide series have been shown to be profound ionic channel blockers in *Loligo* axons. Studies with mercuric ion on the voltage-clamp currents in the squid giant axon are consistent with the hypothesis that sulfhydryl groups are located within the vicinity of the ionic channels. These studies may have great significance: firstly, in understanding the nature of heavy metal poisoning, and secondly, in providing clues for alleviating such blockades.

## Resistances in series with the excitable membrane

The resistances, $R_s$, in series with the membranes of *Loligo* and *Myxicola* giant axons were measured using a constant-current pulse under passive current-clamp. Membrane capacities, $C_m$, were also obtained with this method. Preparations are under way to measure the dimensions of the Geren-Schmitt space, the Schwann cell layer, the basement membrane and the external collagen fiber layer from previously fixed sections of these giant axons. A paper entitled, "Determination of the resistance in series with the membranes of *Loligo* and *Myxicola* axons" is in preparation. In addition, these results have been compared with estimates of $R_s$ and $C_m$ obtained by other methods. These new values of $R_s$ have been extremely useful in voltage clamping axons as they allow for more accurate compensation of these resistances and thus result in more precise determination of membrane ionic conductances. In addition to the experimental determination of $R_s$ an analysis of series resistance, capacitance relaxation and other sources of error in the determination of axon kinetics was performed. One outgrowth of the theoretical study of axon kinetics was the realization that failures in voltage clamp technique could lead to errors in the determination of kinetic parameters. Among these analyses were: (1) the effects of large series resistance values on the form of clamp currents, (2) the axon response with an imperfect membrance capacitance and (3) the series impedance measuring process itself.

## Lipid bilayer membranes

The bilayer laboratory continues as a training ground for visiting scientists. The work on ionophores from ATPase is described elsewhere in this report. The work on excitability inducing material (EIM) has reached the stage where it is mostly concerned with the kinetics of the opening and closing of single EIM channels. As each channel has been shown to be a single EIM molecule these kinetics can be taken as identical to the conformal changes of single EIM molecules. One of the questions examined, concerns whether the two separate negative resistance regions of the EIM channel conductance regime represent two distinct molecular states. It is now clear that the two state case is a reality. This result has led to a theoretical treatment of the expected conformational changes responsible for the two negative resistance states. Upon generalization, this theoretical treatment becomes a model system for sodium channels in real nerve, inasmuch as both have activation and inactivation processes. It is now hoped that this new modelling approach will lead to a replacement of the arbitrary empirical Hodgkin-Huxley equations with equations derived from molecular principles.

## Modelling

A model is a representation of something which cannot be observed directly. Modelling of the behavior of sodium and potassium conductances in nerve continues to be an activity of the Laboratory simply because we have not as yet isolated and chemically characterized the sodium and potassium ion channel molecules found in nerve. As we learn more about these molecules, the model systems begin to describe and predict the presumed molecular reality more accurately. Almost all modelling of the sodium and potassium conductances by the Laboratory of Biophysics now proceeds from the assumption that these measurable conductances are related to ion movement through sparsely located membrane channels which undergo critical conformational changes as a function of the local electric field (membrane potential). The abandonment of the carrier hypothesis and the fleshing in of the channel model has been a direct result of the last decade of voltage clamp experiments on axons. As the Laboratory has done and continues to do much of this voltage clamp work, our models have now progressed to the point where they are beginning to suggest the molecular nature of the sodium and potassium channels in nerve. In great measure, this has also been a result of the extremely valuable clues obtained from the ionophore doped lipid bilayer studies carried on by the Laboratory. None of this work could proceed far without the development of appropriate applied mathematical systems. In this regard, recent developments in dimensional analysis and in statistical mechanical treatments of axon and channel phenomenologies have been of great value. To a large extent the testing of model systems has depended on the intelligent use of digital computers and appropriate programming. In this area, the Laboratory of Biophysics has continued to develop its own potential and acknowledges the help received from the Division of Computer Research and Technology.

Serial No. NDS(I)-62 LB/OC 935
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ionic Permeabilities of Excitable Membranes. Electrical Experiments and Analyses.

Previous Serial Number: Same

Principal Investigators: R.E Taylor, Ph.D. and E. Rojas, Ph.D.

Other Investigators: J. Vergara, Ph.D., R.D. Keynes, Ph.D. and F. Bezanilla, Ph.D.

Cooperating Units: Laboratorio de Fisiologia Celular, Montemar, Chile.

Laboratory of the Marine Biological Association, Plymouth, England.

Department of Physiology, University of Rochester, Medical School, Rochester, New York.

Man Years:

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|---|---|
| Total: | 0.4 |
| Professional: | 0.4 |
| Other: | 0.0 |

Project Description:

Objectives: Interpretation of the function of excitable membranes in terms of ionic permeabilities and electrical impedances and the role of the membrane in depolarization contraction coupling of muscle.

Methods Employed: Single nerve fibers of squid and single muscle fibers of barnacle are mounted in such a way that methods for internal perfusion, voltage clamping and, for muscle, tension recording, can be combined with measurements of radioactive tracer movements. Digital computers are used to assist in the analysis of models to interpret the data.

Major Findings: Analysis of data concerning the influx of calcium and magnesium into the squid axon as effected by membrane potential has continued. The qualitative conclusions are the same as reported last year. Publication of these results is in preparation.

The main results of studies of membrane currents in the barnacle muscle fiber during voltage clamp have been published, viz., non-inactivating calcium and potassium ionic mechanisms. Sufficient calcium appears to enter during the action potential to account for the activation of the contractile machinery. Digital computer analysis of a model for the complicated geometry of this fiber has continued.

Considerable analysis has been done for the results of the studies on the kinetics of the action of saxitoxin and tetrodotoxin on squid axon membranes, directed particularly toward a precise determination of the conductance for a single sodium channel. The cost of computation using the NIH facilities appears prohibitive. Although considerably slower, the PDP-11 in the Laboratory of Biophysics can do the job and is being used.

Some experiments were also done in Plymouth, England, on the selectivity of the sodium and potassium channels to organic cations and these results are being prepared for publication.

Considerable theoretical analyses has been done on various problems of voltage clamp stability and on a modification of the "Lettvin" model for nerve activity.

Significance to Biomedical Research and the Program of the Institute: The movement of calcium ions across cell membranes in response to potential changes appears to be a wide spread mechanism for the activation of a number of physiological processes. These include release of transmitter substances at nerve terminals, initiation of muscular contraction, control of ciliary movement, biolumineserce and response of some retinal rods to light. Any function for a comparable inward movement of magnesium ions is still unknown. The determination of the properties of single sodium, potassium and calcium channels is a major preoccupation of many investigators and is of basic importance.

Proposed Course of the Project: Further experiments with voltage clamped, perfused barnacle muscle fibers are planned. The similarity of the role of calcium in crustacean muscle to that in mammalian heart will be investigated since calcium seems to serve a dual role in each case. In skeletal muscle calcium is involved in contraction activation and may be one of the important steps which are disordered in muscular dystrophy. Further analysis of voltage clamp electronic stability is in progress, as well as the properties of models for the electrical behavior of barnacle muscle fibers. A chapter on "Excitable Membranes" is being prepared for the Annual Reviews of Physical Chemistry.

Honors and Awards: None

Publications:

Keynes, R.D., Rojas, E., Taylor, R.E and Vergara, J.: Calcium and potassium systems of a giant barnacle muscle fibre under membrane potential control. J. Physiol. 229: 409-455, 1973.

Taylor, R.E and Rojas, E.: The right path. In Agin, D.P. (Ed.): Perspectives in Membrane Biophysics, London, Gordon and Breach Science Publishers, Inc., 1972, pp. 1-14.

Serial No. NDS(I)-62 LB/OC 936
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ionic Permeabilities of Nerve Membranes: Theoretical Investigations.

Previous Serial Number: Same

Principal Investigator: R. FitzHugh, Ph.D.

Other Investigators: W.J. Adelman, Jr., Ph.D., K.S. Cole, Ph.D., R. E Taylor, Ph.D., H. Lecar, Ph.D., and J. Shaw, M.A.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 2.3 |
| Professional: | 2.3 |
| Other: | 0.0 |

Project Description:

Objectives: To formulate and test theoretical descriptions of (1) excitable ion-permeable membranes, including statistical fluctuations, and (2) voluntary muscular control, using mathematical analysis and computation.

Methods Employed: Mathematical analyses, with particular emphasis on nonlinear differential equations and optimization theory, are being used in conjunction with the available computing facilities.

Major Findings: Solutions of the Hodgkin-Huxley equations as modified by Adelman and Palti to describe the accumulation of potassium in a space external to the membrane have been obtained using the modeling program MLAB on the PDP-10. Results provide a better fit to recorded action potentials than the unmodified equations do.

Computations of the transient response for a constant phase angle capacitance using a rational approximation have been verified using an integral formula. A paper has been completed and submitted for publication.

The theoretical response of an axon to white-noise stimuli (analyzed by the Wiener theory of nonlinear systems) consists of a subthreshold and a suprathreshold component. The former is equivalent to the impulse response of a linear system, the latter to a stochastic train of action potentials. Parameters characterizing of statistical firing were calculated and agreed well with experimental data of R. Guttman.

Further computations were carried out in an attempt to relate the experimental findings on the effects of anticonvulsant drugs applied to the squid giant axon and the in vivo ability of these drugs to regulate the frequency of nerve firing. Although much agreement with experiment was found for the effects of sodium-blocking drugs on the shapes and thresholds of single action potentials, the modeling of repetitive firing in terms of the Hodgkin-Huxley model has proven problematic. A number of modifications of the Hodgkin-Huxley equations were attempted and none had the desired effect of making the frequency of repetitive firing sensitive to drug-induced parameter changes.

Significance to Biomedical Research and the Program of the Institute: Computation of the transient response of a constant phase angle capacitance facilitates analysis of the capacitative transient of voltage clamp records in the squid axon. The further problem of modeling such capacitances is unsolved, but if accomplished might improve our understanding of the structure of the membrane itself.

The modeling of the extracellular potassium space of the squid giant axon, for comparison with recent experimental data, can be expected to provide more definite evidence for its existence and properties. Since the accumulation of potassium is a slower process than those involved in excitation and recovery, it is important to find out whether it can explain adaptation, change of frequency or cessation of a train of impulses following a constant current stimulus, which is absent from the Hodgkin-Huxley equations. The statistical analysis of firing of axons should be useful in relating two types of independent experimental endeavor — analysis of membrane currents and analysis of spike trains.

Proposed Course of the Project: The study of both membrane stability and membrane fluctuations will be pursued, with the aim of improving present mathematical models of the excitable membranes, which are inadequate in these respects.

The computation of solutions of the equations for optimal muscular control when a series elastic element is included has proved to be a more difficult numerical problem than expected. Further approximation methods will be tried in an attempt to include this important case, before publication of results already obtained. Further theoretical study of the factors influencing repetitive firing is planned in conjunction with experiments on giant axons by Gilbert and Lecar in conjunction with Gilbert-Lipicky experimental work.

Honors and Awards: None

Publications:

Cole, K.S.: Biophysica and a nerve impulse. In Chagas, C. (Ed.): Three Lectures on Biophysics, Rio de Janeiro, Instituto de Biofisica, 1972, pp. 15-26.

FitzHugh, R., and Rapoport, S.I.: Stress-strain properties of the elastic cylinder. Biophys. J. 13: App. I 30-33, 1973.

FitzHugh, R.: Dimensional analysis of nerve models. J. Theoret. Biol. In Press.

FitzHugh, R. and Cole, K.S.: Dielectric loss and voltage clamp transients. Biophys. J. In Press.

Serial No. NDS(I)-62 LB/OC 938
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Membrane Dielectric Properties.

Previous Serial Number: Same

Principal Investigators: K.S. Cole, Ph.D. and R.E Taylor, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 0.7 |
| Professional: | 0.6 |
| Other: | 0.1 |

Project Description:

Objectives: I. The need for more and detailed measurements of membrane dielectric properties remains, particularly in the low frequency range, for the effects of temperature and drugs and effect of membrane potential.

II. To understand the amount and nature of dielectric loss in the membranes of isolated and tissue cells.

Methods Employed: I. The presently available methods for space and voltage clamping the squid giant axon are required as well as the developing techniques for internal replacement and perfusion, the use of accurate bridge networks, and powerful analysis.

Major Findings: I. Squid membrane dielectric information depended upon the almost ancient alternating current external electrode measurements and the relatively crude transient and other direct records until Chandler and Taylor made bridge measurements from 10 kHz to 70 kHz between coaxial internal and external platinized electrodes. Similar measurements have been made by Matsumoto, et al., from 50 Hz to 1MHz with changes of K in the external ASW. Takashima, et al., have made a preliminary report of extensions of their 1971 work.

Alternative analyses of these data are not complete but they seem to rather well agree in assigning a passive lossy dielectric characteristic of $10^{\circ} - 15^{\circ}$ phase to the membrane above 10 kHz. Suitable techniques have not yet been found for representing and characterizing the behavior at lower frequencies at which the ionic conductance relaxations may dominate.

Significance to Biomedical Research and the Program of the Institute: Detailed information on the impedance of the membrane, particularly the voltage and temperature dependence relations, imposes constraints on any molecular model which is proposed. One example is that the membrane does not have the same dielectric properties as the simple bilipid layers which have been worked on. Preliminary unpublished data from Mueller indicate that artificial bilayers formed from dissimilar monolayers show appreciable dielectric loss seem highly significant.

Proposed Course of the Project: No further experimental work on this aspect is currently planned.

Honors and Awards:

Kenneth S. Cole was formally admitted as a Foreign Member of the Royal Society at the 312th Anniversary Meeting in London, November 30, 1972.

Kenneth S. Cole was honored by the publication of a volume edited by Dr. D.P. Agin entitled, "Perspectives in Membrane Biophysics - A Tribute to Kenneth S. Cole", published in 1972.

Publications: None

Serial No. NDS(I)-62 LB/OC 939
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ionic Permeabilities of the Squid Giant Axon Membrane. Electrical Experiments and Analyses with Alterations of Environments.

Previous Serial Number: Same

Principal Investigator: D.L. Gilbert, Ph.D.

Other Investigators: R.J. Lipicky, M.D., I.M. Stillman, M.D., G. Ehrenstein, Ph.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

Man Years:

| | |
|---|---|
| Total: | 1.3 |
| Professional: | 1.2 |
| Other: | 0.1 |

Project Description:

Objectives: To study the ionic current flow across the nerve membrane without the complications of excitation and propagation in terms of individual ion currents and to observe the effect of changes in normal internal and external ionic concentrations. The long range objectives are the interpretation of nerve function in terms of ion movements and the elucidations of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Standard voltage clamp techniques are employed with the addition of control of the internal environment by replacement and perfusion.

Major Findings: We have continued our studies with diphenylhydantoin. Previously, we have shown that diphenylhydantoin decreased the sodium, and to a lesser extent the potassium, currents of the voltage-clamped squid giant axon. If concentrations of diphenylhydantoin of the same magnitude are applied internally, the time course of the inhibition of the sodium conductance is similar to that obtained when diphenylhydantoin is applied externally. The internally applied diphenylhydantoin also inhibited the potassium currents.

The role of possible sulfhydryl groups on the ionic currents were also studied. We confirmed earlier studies performed by others, which showed that mercuric ion blocks the action potential and that the blockage can be reversed by addition of sulfhydryl containing compounds. In voltage-clamp experiments, mercuric acetate greatly decreased the sodium currents. Removal of the mercuric acetate with the addition of a sulfhydryl containing compound, 2 mercaptoethanol, resulted in partial recovery of the sodium currents. The potassium currents were also markedly decreased by the mercuric ion.

Analysis of the time course of the potassium currents obtained from voltage-clamped axons immersed in high potassium solutions revealed that the potassium time constant-potential curves were shifted along the potential axis as a function of the divalent cation concentration. This shift was quantitatively the same as the potassium conductance-potential curves obtained under the same conditions.

Significance to Biomedical Research and the Program of the Institute: Diphenylhydantoin, one of the principal agents used in the treatment of epilepsy, affects the sodium currents whether applied internally or externally in much the same manner. However, the potassium currents seem to be principally affected only by the internal perfusion of this agent.

Our mercuric ion studies indicate that this ion is binding to membrane sulfhydryl groups within the vicinity of the ionic channels.

The shift of the potassium time constant-potential curves along the potential axis gives further support for the notion that there is a negative surface charge on the external nerve membrane in the vicinity of the potassium channels.

Proposed Course of the Project: It is planned to study further the effect of pharmacological agents and trace metals upon the voltage-clamped currents of the squid giant axon.

Honors and Awards: None

Publications:

Gilbert, D.L.: Introduction: Oxygen and life. Anesthesiology 37: 100-111, 1972.

Lipicky, R.J., Gilbert, D.L., and Stillman, I.M.: Diphenylhydantoin inhibition of sodium conductance in squid giant axon. Proc. Natl. Acad. Sci. USA 69: 1758-1760, 1972.

Ehrenstein, G., and Gilbert, D.L.: Evidence for membrane surface charge from measurement of potassium kinetics as a function of external calcium concentration. Biophys. J. In Press.

Gilbert, D.L.: Potential and time constant effects in the fast voltage clamp. Math. Biosci. In Press.

Serial No. NDS(I)-62 LB/OC 940
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ionic Permeabilities of Excitable Membranes: Effects of Chemical Agents.

Previous Serial Number: Same

Principal Investigator: L. Binstock, B.S., M.S.

Other Investigators: R. DiPolo, M.D., and R. Latorre, Ph.D.

Cooperating Units: Division of Computer Research and Technology, NIH

Section on Technical Development, NIMH/NINDS, NIH

Instituto Venezolano de Investigaciones Cientificas,
Caracas, Venezuela.

Faculty of Sciences, University of Chile,
Santiago, Chile.

Man Years:

| | |
|---|---|
| Total: | 1.8 |
| Professional: | 0.7 |
| Other: | 1.1 |

Project Description:

Objectives: To study the conducting pathways in nerve membrane by means of an analysis of the action of various chemical agents with these pathways. The ionic current flowing across these pathways is measured by means of the voltage clamp technique. Why the movement of a particular ion through one pathway or another or even both is not known. It is possible that the use of various chemical agents that affect the nerve membrane may contribute to understanding the physical mechanisms of the membrane. One possibility is the study of various ions to determine their selectivities through the membrane. Another is to block the conducting pathway or alter the kinetics.

Methods Employed: Previously described voltage clamping techniques are used, either with or without perfusion, with the giant axon of the marine worm, Myxicola infundibulum. For perfusion, a cannula is first inserted into the axon and passed right through the axon to the other side of the chamber, removing a good part of the axon. Sometimes the axoplasm is very gelled and it is difficult to remove a good part of the axoplasm, instead the cannula pushes the axoplasm aside. At times it is necessary to use different enzymes such as pronase, cystein or trypsin to soften the gelled axoplasm. Pressure is then applied to the perfusate forcing out the axoplasm which has accumulated during the movement of the cannula through the axon. It may be necessary to apply suction at times to assist in the removal of the axoplasm. As soon as flow is established, the axial wire of the "piggyback" electrode (combination current supply and voltage measuring device) is inserted into the perfusion cannula which acts as a guide. The cannula is then slowly withdrawn close to its entry point and at the same time, advancing the piggyback electrode into the axon. Some axoplasm is removed by this method and replaced with perfusate. Finally, the axon is tied just beyond the entry point of the cannula to prevent the perfusate flowing out the entry point.

The data is obtained with the Micro-LINC 300 for possibly both on and off line analysis of the voltage clamped axons. Data sampling time is routinely 40 or 10 micro-seconds with a 1.0 microsecond capability. The Micro-LINC 300 is available only on a part-time basis. Data from LINC tape can be sent via the PDP-12 to the PDP-10 at DCRT for additional processing. LINC tapes can also be converted on the PDP-12 for use on the PDP-10 and taken to DCRT for processing. Complete reduction of data by computer is still not completely worked out. The PDP-10 MLAB works well for certain parameters but extension to MLAB are being worked out for economical and efficient use. A program must be able to handle the variability in the data produced by the Myxicola giant axon.

Major Findings: The resistance, $R_s$, in series with the membrane of the Myxicola giant axon was measured using a constant-current pulse under passive current-clamp. Membrane capacity, $C_m$, was also obtained with this method. $R_s$ as measured from the capacity current transient under voltage-clamp was in agreement with that obtained under current-clamp. Simulated tests were made with a dummy axon for a range of series resistance values. Results obtained were very good. Dual oscilloscope tracings, one of the voltage response and the other the constant current pulse at a resolution of 1 mv/cm and 2μsecond/cm were obtained. The voltage response was obtained from the internal probe and the reference electrode. Series resistance measurements were very quickly made from polaroid photographs of the oscilloscope tracings. For 22 axons the total series resistance, $R_{s\ total}$ is $8.41 \pm 1.36$(S.D.)ohm cm$^2$, $R_{residual}$ is $3.275 \pm 1.04$(S.D.)ohm cm$^2$ and $C_m$ is $1.245 \pm 0.25$(S.D.)μF/cm$^2$. $R_{residual}$ is that resistance remaining after axoplasmic and sea water resistances are subtracted from the total series resistance. The residual resistance is the resistance of the extra-cellular space. Preparations are under way to measure the thickness of this extracellular space from previously fixed sections of the Myxicola giant axon. $R_s$ computations were made with MLAB using Hodgkin-Huxley simulations.

A total series resistance of over 8 $\Omega\ cm^2$ can produce errors sometimes large if large currents are available. This resistance can be corrected for by compensated feedback. In the study of the effects of various chemical agents on the membrane, erroneous results may be obtained. For example, the total series resistance of the membrane with $D_2O$ applied externally is about 11 $\Omega\ cm^2$, this being almost enough to negate the shift in the steady state inactivation reported previously. It is therefore important for Myxicola axons to reduce the affect of the membrane series resistance so that errors are reduced to a minimum and comparisons between control responses and the effects produced under different conditions can be made.

Several more cations have been substituted for sodium to see the effect on the ionic permeabilities. These were methylated cations such as methylamine and acetamidine and proved to be relatively impermeant.

Acetate sea water has been used to make it easier to get lead into solution. Acetate, substituting for chloride, depolarizes the axon 4-7 mv, reduced the action potential 30-50% and reduces the inward and outward currents by approximately 50%. There is also a decrease in the leakage currents with acetate. With $10^{-3}$ lead acetate added to the acetate sea water there is a further decrease in the ionic currents (Na and K), slower time constants and a decrease in the leakage currents.

Scorpion venom at a concentration of 18.4 mg/liter of normal sea water produced no changes in the ionic currents or in the action potential. Scorpion venom is reported to slow the action potential many fold as well as delay the early currents. The difficulty encountered is that scorpion venom is not readily soluble in sea water and concentration may be much less than we think it is.

Sea Nettle Toxin (SNTX) at higher strengths (up to 80 mg/liter) showed very little change in the ionic currents. SNTX has now been prepared by another method similar to that used initially on squid axons and it will be tried again before the end of this report period.

Various concentrations of Saxitoxin (STX) have been tried. The Myxicola axon tolerates STX much better than tetrodotoxin (TTX), recovering better and quicker. With the information obtained it should be possible to determine the number of Na sites per given area.

Amiloride, a powerful diuretic, specific for sodium, at a concentration of $10^{-4}$ greatly reduced the ionic currents irreversibly.

Internal perfusion still poses a problem even with large axons. Various enzymes have been tried, different techniques including suction and different ways of applying pressure to the infusion cannula. For example, l-cystein completely liquifies extruded axoplasm in about 4 minutes. However, one doesn't know what happens to the membrane. It is possible to obtain fairly good action potentials at the initial insertion of the electrode, but once everything is in place the action potential becomes small or disappears. With low resting potentials, it is possible to obtain ionic currents with high holding potentials.

The temperature-dependent component of the resting potential in intact, cannulated and dialysed fibers from the muscle of the barnacle Balanus nubilus was studied under a variety of different experimental conditions. A decrease in temperature from 22 to 12°C produced a mean depolarization of 10 mV. Neither addition of strophantidin, nor replacement of external sodium by lithium affect the voltage shift induced by temperature. However, the magnitude of the voltage shift depends on the external chloride and potassium concentration. The dialysis technique was applied to measure the potassium, chloride and sodium fluxes as a function of temperature. The $Q_{10}$ for the passive fluxes of these ions as 1·9, 1·7, and 1·4 respectively. The temperature-dependent changes in the passive ionic fluxes combined with the inability of inhibitors of the sodium pump to alter the temperature dependence of the resting potential suggest that the change induced by temperature on the resting potential is primarily caused by a change in the passive permeability ratios, and is not related to active ion transport.

Significance to Biomedical Research and the Program of the Institute: The sizes of the various permeant ions might give the dimension of the conducting pathway. The interactions of the various chemical agents with the axon may produce a pharmacological effect. Under these conditions, it appears that the nerve membrane may be altered. Any changes in the axon membrane might possibly give information as to the site of the particular conducting pathway. Therefore, these studies on the Myxicola giant axon are making it possible to get some kind of insight into the mechanism of the excitable membrane. Studies on the environmental pollutants may yield important information which can be of great value in clinical applications.

Proposed Course of the Project: The study of "sodium substitutes" and "potassium substitutes" of the quaternary-ammonium ions and their various analogs and others will be continued combined with chemical analysis of the axon membrane. $D_2O$ should be completely finalized by the beginning of the next report period. Batrachotoxin (BTX) will be studied again as soon as available. Sea Nettle Toxin, scorpion venom, environmental pollutants (lead studies) and comparison of Saxitoxin with TTX will be continued. It is planned to study other chemical agents such as anesthetics, tannic acid, and pronase. Internal perfusion will be continued with voltage clamping. Injection may be substituted if perfusion continues to pose a problem. The excitable membrane to be used is the giant axon of Myxicola.

Honors and Awards: None

Publications:

DiPolo, R., and Latorre, R.: Effect of temperature on membrane potential and ionic permeabilities in barnacle muscle. J. Physiol. (Lond) 224: 1-19, 1972.

Serial No. NDS(I)-65 LB/OC 1240
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Molecular Biophysics - Physical Properties of Membranes and Simple Membrane-Like Systems.

Previous Serial Number: Same

Principal Investigators: G. Ehrenstein, Ph.D., H. Lecar, Ph.D., A. E. Shamoo, Ph.D., R. Blumenthal, Ph.D.

Other Investigators: R. W. Albers, Ph.D., M. Myers, B.S., M. Lincoln

Cooperating Units: Laboratory of Neurochemistry, NINDS, NIH

Laboratory of Theoretical Biology, NCI, NIH

Man Years:

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|---|---|
| Total: | 3.6 |
| Professional: | 2.2 |
| Other: | 1.4 |

Project Description:

Objectives: To study the physical mechanisms for ion transport in synthetic membranes. To develop the lipid bilayer membrane as an assay for ion-conductance inducing materials isolated from natural cell membranes. The ultimate goal of the synthetic membrane studies is the elucidation of the molecular mechanism of membrane excitation and active transport.

Methods Employed: Synthetic bilayer membranes are formed from natural membrane extracts, oxidized cholesterol, or pure lipids in an aperture between two electrolyte solutions. The steady state and fluctuating electrical properties of the membranes are measured before and after the application of an activating factor, such as EIM (a bacterial protein which induces excitability). The kinetic properties of EIM-DOPED membranes are obtained primarily from the study of the electrical fluctuations which accompany molecular rearrangements within the membrane. The study of active transport involves the biochemical isolation of a $Na^+$-specific ionophore from Na+K-ATPase and biophysical measurements of the effect of the ionophore on lipid membranes.

Major Findings: The kinetics of EIM-induced conductance changes were studied by the method of analysis of discrete conductance fluctuations. The EIM conducting channels were shown to obey Poisson statistics with transition rates which vary in a simple exponential manner with the transmembrane electrical potential. The measurements of microscopic conductance fluctuations were shown to be completely consistent with the macroscopic parameters determined from voltage clamp experiments. This complete specification of the kinetic parameters of a system of ion conducting channels of molecular dimensions has allowed us to determine the nature of the energy barriers involved in the activation of the excitable units.

We have isolated an ionophore from electrophorus electric organ and beef Kidney membrane fractions which is activated only in the presence of $Na^+$ ions. The $Na^+$-ionophore was isolated from the acid soluble fraction of a tryptic digest of membraneous fractions. The test for the $Na^+$-ionophoric property was demonstrated by the action of the peptidic material on black lipid membrane assay system. The conductance change elicited by this material is specifically activated by $Na^+$. The activity of the material was greatly enhanced by passage through DEAE-cellulose. Activity could be destroyed by further incubation with pronase. Since conductivity increases exponentially with dose of ionophore, the conductive unit may be an oligomer. The molecular weight of the monomeric units of the active material is estimated to be ∿ 2000. The dissociation of the active material into monomers renders the material inactive. We were also able to isolate the $Na^+$-ionophore from Lubrol soluble Na+K-ATPase of the eel electroplax. The Lubrol soluble Na+K-ATPase contains mainly two protein bands on SDS gel electrophoresis. This increases the functional relationship between the enzyme Na+K-ATPase and the Na-ionophore.

Significance to Biomedical Research and the Program of the Institute: The analysis of single conducting channels in the EIM doped bilayer has given support to the idea of discrete conducting channels in excitable membranes. The methods developed in this laboratory have now been extended to other excitability-inducing materials, and unit conducting channels have been demonstrated for several substances. Study of discrete electrical fluctuations is a sensitive means of determining the kinetics of membrane transport. The voltage-dependent permeability properties of the synthetic excitable membranes bears many similarities to those of natural excitable membranes. The bilayer has proven to be an exquisitely sensitive probe for the molecular properties of ion conducting channels in membranes.

The extension of the bilayer techniques as an assay for the ionophore fractions of Na-K ATPase offers the promise of the reconstruction of such membrane processes as active transport on a synthetic membrane.

The maintenance of Na-K gradient in nerve cell between inside and the outside of the cell requires an intricate active transport system of $Na^+$.

Without the Na-K gradient, nerve cells cannot sustain action potential and nerve transmission. The enzyme Na+K-ATPase has been known to provide the energy source for active transport of Na by splitting ATP molecule. The search for the Na-site of action in the membrane has been without results. Our data suggest that the specific site of Na-site is an integral part of the enzyme and may provide the first step in the elucidation of the energy transduction from splitting ATP molecule to Na-ion.

Proposed Course of the Project: We are in the process of writing a series of papers about the EIM conductance-change kinetics. The first paper is concerned with the relation between the single-channel fluctuations and the microscopic conductance transitions. A second paper in preparation is concerned with more general aspects of conductance-change kinetics. Experiments are planned to measure the nonelectrolyte flux in EIM channels.

The ATPase ionophore is being studied further. Experiments are in progress to determine the ionic specificity of the active fraction and whether the ionophore acts as a carrier or a channel within the membrane.

Honors and Awards:

H. Lecar was invited to review the area of "Excitability in Bilayers" at the Membrane Subgroup Symposium of the Biophysical Society.

G. Ehrenstein was invited to preside over the Synthetic Membrane Symposium at the 1973 Gordon Conference.

Publications:

Latorre, R., Ehrenstein, G., and Lecar, H.: Ion transport through excitability inducing material (EIM) channels in lipid bilayer membranes. J. Gen. Physiol. 60: 72-85, 1972.

Shamoo, A., Albers, R. W.: Na-selective ionophoric material derived from kidney and electric organic membranes. Proc. Natl. Acad. Sci. USA. In Press.

Serial No. NDS(I)-72 LB/OC 1947
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cytoplasmic Water Structure.

Previous Serial Number: Same

Principal Investigators: F. Sachs, Ph.D., and R. Latorre, Ph.D.

Other Investigators: H. Lecar, Ph.D., D.O. Carpenter, Ph.D.

Cooperating Units: Neurobiology Department, Armed Forces Radiobiology Research Institute, DNA.

Man Years:

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|---|---|
| Total: | 0.3 |
| Professional: | 0.3 |
| Other: | 0.0 |

Project Description:

Objectives: (1) To see whether the bulk of cell water in barnacle muscle fibers is ordered on a time scale to which the EPR is sensitive, 20 psec to 100 nsec.

(2) To investigate axoplasmic conductivity utilizing the technique for measurement previously developed, and to determine the effects of frequency variation, temperature and axonal size on conductivity.

(3) To determine the specific membrane resistance of somal membranes and the possible role of an electrogenic $Na^+$ pump in generation of membrane potential in the cell bodies.

Methods Employed: (1) The basic techniques for introducing the probe were developed last year. This year's research involved the effect on temperature on the EPR spectrum of the probe in cells and model systems. Probe containing systems were temperature cycled in the Varian E3 EPR spectrometer using the variable temperature accessory supplied with the machine.

(2), (3) The technique of conductivity measurement was reported in Internat. J. Neurosci. 2: 35, 1971. Modifications were only so as to allow conductivity to be measured while sweeping the frequency of applied current from 25 to 250 kHz. Intracellular recordings from cell bodies were made from the stellate ganglion pinned to a leucite chamber and maintained under flowing sea water. Temperature was varied by passing the inflow through an ice bath. Resistance was measured by passing current through the microelectrode in a conventional bridge circuit.

Major Findings: (1) In the previous year, we showed that the probe sampled the bulk of cell water, and that a fraction of the probes present became immobilized with 25% of cell water remaining. Activation energies for the tumbling of the radical in fully hydrated muscles and model monomeric and polymeric solutions were measured. Aqueous solutions of simple solutes and gelatin showed activation enthalpies of 8 to 11 kcal/M, and activation entropies of 20 eu. The radical correlation time in these solutions was a monotonic function of the activation enthalpy. In the muscle, 20% bovine serum albumen, and the giant red cells of Amphiuma the probe yielded biphasic Arrhenius plots indicating the presence of multiple processes. The simplest explanation was that the label was undergoing fast exchange between two or more environments, and the temperature dependence reflects not only the tumbling of the probe, but also the redistribution of probes between the two environments.

(2) The average axoplasmic conductivity obtained from those stellar axons studied was 39 ohm cm, equivalent to 65% sea water. Smaller axons showed lower conductivities (45 ohm cm). The electrode with changing frequency behaves like a resistor and capacitor in series both in calibrating solution and in the axons. Conductivity of axoplasm varies with temperature as does the conductivity of an equally conductive salt solution.

(3) A portion of resting membrane potential in squid somata (up to 20 μ) is abolished by cooling and ouabain. On this basis, we conclude that it is produced by an electrogenic $Na^+$ pump. However, there may be no essential differences between rate of $Na^+$ transport between axon and soma, since the efficacy of the pump can be explained by a difference in membrane resistance. The specific membrane resistance of axons is about 1000 ohm $cm^2$ but that of the soma is about 30,000 ohm $cm^2$, as determined both from the membrane time constant and from estimations of surface area and input resistance. This work was reported in Science 179: 1336, 1972.

Significance to Biomedical Research and the Program of the Institute: (1) The temperature studies indicate that the probes are temporarily bound and rapidly exchanging with free probes. This means that the average viscosity figures measured last year for the muscle (5cp) are lower bounds, and thus the free component of the probe is in an environment with a viscosity similar to pure water. There appears to be no long range ordering of cell water. The fast exchange model for the probes behavior may also be applicable to the cell components, in particular, ions, and may explain Dr. Carpenter's low conductance values bound in the barnacle muscle and other cells.

(2), (3) These studies are aimed at approaching problems of basic importance in the understanding of nerve cell function. Once we understand how nerve cells behave under normal conditions, we can hope to apply this information to diseased nerve cells.

Proposed Course of the Project: The original goals have been fulfilled and we are ending active research on the project.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-72 LB/OC 1948
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electrical Properties of Small Patches of Excitable Membrane.

Previous Serial Number: Same

Principal Investigators: H. Lecar, Ph.D., F. Sachs, Ph.D., and G. Ehrenstein, Ph.D.

Other Investigators: None

Cooperating Units: The Technion, Haifa, Israel

National Institute of Child Health and Human Development, NIH

Man Years:

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|---|---|
| Total: | 1.1 |
| Professional: | 1.0 |
| Other: | 0.1 |

Project Description:

Objectives: The object of this project is to record ionic currents from small electrically isolated patches of cell membrane. Such a procedure allows one to perform voltage clamp experiments without the use of internal electrodes and to probe the surface of a cell membrane for isolated ion conducting regions. The major use of this type of recording will be to study membrane conductance fluctuations. The analysis of fluctuations in membrane currents provides a means for determining the elementary conductance unit (as has been demonstrated in our experiments on synthetic membranes). Fluctuation analysis can be used to study the kinetics of the molecular processes which cause membrane excitation.

Methods Employed: The major problem to be overcome is the means of isolating a region of membrane of area 10 to 100 square microns. This necessitates the design of a novel electrode in which the current recording aperture is sealed off from the bathing medium of the tissue. Recordings are made from nerve and muscle cells grown in tissue culture. These preparations were chosen both because of their intrinsic interest and because they present a relatively naked plasma membrane unencumbered by glia or connective tissue.

The studies on different preparations employ low-noise amplification techniques and computer analysis of the recorded electrical fluctuations.

Major Findings: The major effort has been to develop the electrode for isolating a small membrane patch. A modernized version of the Pratt (1917) electrode-fabrication technique has been developed, which employs a servomechanism driven grinder to produce shaped electrodes of micron dimensions with apertures whose size can be controlled. Scanning electron microscope pictures show the electrode to have the desired geometry for a membrane seal. Preliminary tests have been performed on neuroblastoma cells.

Significance to Biomedical Research and the Program of the Institute: Fluctuations in membrane currents provide a means of identifying the discrete transitions of ion conducting structures responsible for membrane excitation. These structures are too sparsely distributed within the membrane to be amenable to study by conventional spectroscopic techniques. However, the electrical current through a single conducting unit may be quite intense and the observation of these minute unit electrical changes provides a sensitive probe for the molecular events underlying excitation. In addition, the current through a sealed patch of membrane may allow the extension of the voltage clamp method to small cells which have elluded quantitative study. Studies on tissue culture preparations, such as neuroblastoma, may be of significance in understanding the development of membrane excitation.

Proposed Course of the Project: The basic recording system is being developed. Recordings will be taken from various tissue culture preparations and computer programs are being written to analyze the electrical fluctuations. Work is being continued on the basic theory relating electrical fluctuations to various proposed models of the excitation process.

Honors and Awards: None

Publications:

Ehrenstein, G., and Lecar, H.: The mechanism of signal transmission in nerve axons. In Morales, M.F. (Ed.): Annual Review of Biophysics and Bioengineering, Palo Alto, Calif., Annual Reviews Inc., 1972, Vol. 1, pp. 347-368.

Lecar, H.: How membrane permeabilities change. In Behnke, J.A. (Ed.): Challenging Biological Problems: Directions Toward Their Solution, New York, Oxford Press, 1972, Vol. 25, Chap. 4, pp. 62-81.

Serial No. NDS(I)-72 LB/OC 1949
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Fluorescence Probes of Activity Phenomena.

Previous Serial Number: Same

Principal Investigators: F. Sachs, Ph.D., and W.J. Adelman, Jr., Ph.D.

Other Investigators: None

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

Man Years:

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|---|---|
| Total: | 0.6 |
| Professional: | 0.5 |
| Other: | 0.1 |

Project Description:

Objectives: To see whether the conductance mechanisms of nerves can be studied on a molecular scale using fluorescent probes.

Methods Employed: In the previous year a high efficiency spectrophotometer was designed and built. When tested in Woods Hole, Mass., on squid axon deficiences on the apparatus became apparent and the equipment had to be redesigned.

Major Findings: The problems with the original apparatus were too low an over-all light flux due to the difficulty of coupling the arc lamp to the objective, and too high a flux density resulting in unanticipated photooxidation of the fluorescent probe. Redesign of the equipment along lines already reported in the literature yielded acceptable records for a number of probes. These probes were sensitive to either membrane potential or integrated current, but none were conductance sensitive.

Significance to Biomedical Research and the Program of the Institute: No substantiated reports of conductance sensitive dyes have appeared, and although the method has promise in principle, a rational procedure for dye selection and synthesis will have to be undertaken before the method is of any value in determining conductance conformational changes.

Proposed Course of the Project: This project has been terminated.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-72 LB/OC 1950
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Rapid Analysis of Excitable Membranes by Means of Computer Controlled Voltage Clamp.

Previous Serial Number: None

Principal Investigators: W.J. Adelman, Jr., Ph.D., L. Binstock, B.S., M.S. and J. Shaw, B.S., M.A.

Other Investigators: James Bryan, M.S.
William Sherriff, M.A.
Fred Sachs, Ph.D., Brad Cox, Ph.D.
Herbert Walters

Cooperating Units: Section on Technical Development, NIMH/NINDS

Man Years:

| | |
|---|---|
| Total: | 1.6 |
| Professional: | 1.0 |
| Others: | 0.6 |

Project Description:

Objectives: To study excitable membranes in a very rapid and precise manner by a program controlled voltage clamp. In this way, it is possible to go through various pulses necessary to determine the desired parameters and while solutions are being changed, on-line analysis may be of extreme value in determining the next step of the experiment. It is also of extreme value, for example, in the study of various chemical agents and their effects on the membrane because fast accumulation of the desired data is obtained, thus allowing for minimum exposure to the particular chemical agent which in many instances would present deleterious effects on the nerve membrane. Some of the parameters that can be obtained are: (1) reversal potentials ($E_{Na}$, $E_K$ and $E_L$), (2) conductance maxima ($\bar{g}_{Na}$, $\bar{g}_K$, $\bar{g}_L$), (3) steady state values of membrane conductance parameters as a function of voltage ($m_o$, $h_o$, $n_o$, $m_\infty$, $h_\infty$, $n_\infty$), (4) time constants ($\tau_m$, $\tau_h$, $\tau_n$), and/or (5) the voltage dependent membrane rate constants ($\alpha_m$, $\beta_m$, $\alpha_h$, $\beta_h$, $\alpha_n$, $\beta_n$).

Methods Employed: PDP-11 programming currently in progress will use the Walters-Bryan interface to control pulse sequences and sampling rates in these experiments and to record the resulting data. The PDP-11 system will display these data on a scope and/or plot them on paper, and store them on magnetic disk or tape. At least some of the analysis of the data will be carried out by the PDP-11, both during and after an experiment. A general aim will be to make use of fast analysis during an experiment to decide some aspects of the course of that experiment itself. The data recorded can readily be used as input to DCRT computer systems when analysis requiring their greater power is needed.

A series of Laboratory staff meetings in Fiscal Year 1972 resulted in general agreement that the Laboratory had a need for a Data Acquisition System capable of rapidly acquiring, storing and processing data, while performing intricate experiment control functions. A study of acquisition systems available led to the conclusion that a system based on a general purpose mini-computer was the most practical and economic solution. Immediate Laboratory needs dictated the initial criteria that the system be capable of acquiring data at high rates (up to $10^6$ samples/second) while producing precision control pulses. Funding restrictions ruled out custom interfacing; therefore, the design and fabrication of the acquisiton and control circuitry was undertaken by the Laboratory with technical assistance being supplied by the Section on Technical Development, NIMH/NINDS.

The selection of a Digital Equipment Company PDP 11/20 computer system to provide the data control and handling functions for the high speed D/A's was formalized in May, 1972. Prior to delivery, preliminary design studies were undertaken to determine system performance criteria. Upon delivery of the equipment (November), engineering design of the interface, pulsing and conversion circuitry was initiated by Mr. H. A. Walters, in conjunction with Mr. James Bryan, Head, Section on Technical Development, NIMH/NINDS.

The resulting design has been fabricated and, with the exceptions of the pulsing and conversion equipment, permanently installed in the computer. All hardware installation will be completed prior to the end of the Fiscal Year and preliminary testing indicates that the system design criteria will be met. Final testing and usage is dependent upon software availablility.

Design criteria required that the system be capable of supplying precision pulses of variable duration and amplitude while simultaneously acquiring data at variable acquisition rates. As designed, the system has 12 data acquisition rates, the fastest being one megahertz, (obtained from a crystal controlled oscillator) the eleven additional rates are obtained by binary division of the clock frequency, the slowest being one sample each 4096 μ seconds. Rates may be changed several times during a pulse sequence. Bipolar pulses are supplied by a high performance D/A converter with a basic accuracy of $10V/2^{10}$, whose duration is determined by number of samples taken. The number of pulse sets, parameters of each set, and spacing between sets are software functions since system operation is entirely computer controlled when the start command is issued. Data storage will normally be on magnetic tape.

Major Findings: This study has obtained additional values of the Hodgkin and Huxley conductance parameters for the *Myxicola* giant axon membrane. However, additional experiments and computer computations are necessary to state with certainty the absolute values of these parameters which have been successfully extracted from the data by the PDP-10 and PDP-10 MLAB. When more runs are added to the present data, the first full description of *Myxicola* axon conductances will have been obtained by a computer program controlled voltage clamp. From these values, the nerve impulse can be reconstructed and excitation and conduction in this preparation can be predicted.

Significance to Biomedical Research and the Program of the Institute: The use of the computer program controlled voltage clamp enables one to quickly collect data and do many experiments in a short time. The analysis of the data obtained can be analyzed on-line or taken to the PDP-10 for analysis. The turn over time from experiment to final analysis is reduced many-fold. Manual control of voltage and data analysis by conventional means is very, very, time consuming. The output of information by this method is increased manyfold and information on the structure and mechanism of the membrane can thus be obtained much sooner than by any other method.

Proposed Course of the Project: The computer program controlled voltage clamp will continue to be used in greater detail in the studies of various membranes both here and at Woods Hole. Many difficult experiments that have not been possible before can now be done in great detail. We expect that most voltage clamp experiments in the future will use the computer controlled technique.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-72 LB/OC 1951
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Conductance Regimes in the Membrane of Giant Axons of *Myxicola infundibulum*.

Previous Serial Number: Same

Principal Investigator: W.J. Adelman, Jr., Ph.D.

Other Investigators: M.D. Goldfinger, M.S.

Cooperating Unit: Department of Physiology, School of Medicine, The University of Maryland, Baltimore, Md.

Man Years:

| | |
|---|---|
| Total: | 0.0 |
| Professional: | 0.0 |
| Other: | 0.0 |

Project Description:

This project has been terminated.

Serial No. NDS(I)-72 LB/OC 1966
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Function and Structure of Membrane Ionic Channels Obtained from Studies Involving Ion Interactions with the Membranes of Giant Axons.

Previous Serial Number: Same

Principal Investigator: W. J. Adelman, Jr., Ph.D.

Other Investigators: J. P. Senft, Ph.D., M. Starzak, Ph.D., and Y. Palti, M.D., Ph.D., B. Cox, Ph.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

Department of Biology, Juniata College, Huntingdon, Pa.

Department of Chemistry, State University of New York at Binghamton, Binghamton, New York.

Department of Physiology and Biophysics, The Aba Khoushy School of Medicine, The Technion--Israel Institute of Technology, Haifa, Israel.

Man Years:

| | |
|---|---|
| Total: | 1.2 |
| Professional: | 0.9 |
| Other: | 0.3 |

Project Description:

Objectives: To determine the behavior of excitable membrane channels as a function of ionic species and concentrations. To relate this behavior to the molecular basis for the nerve impulse. To predict axon and neuron responses from models derived from these studies.

Methods Employed:

1) Modification of ionic flow across neural membranes through ion concentration changes in periaxonal and perineuronal spaces resulting from membrane activity. Single giant axons from the squid, *Loligo pealei*, were prepared and voltage clamped by methods described in previous reports.

Potassium currents of various durations were obtained from squid giant axons voltage clamped in artificial sea water solutions containing sufficient tetrodotoxin to block the sodium conductance completely. From instantaneous potassium current voltage relations, the reversal potentials immediately at the end of these currents were determined. On the basis of these reversal potential measurements, the potassium ion concentration gradient across the membrane was shown to decrease as the potassium current duration increased. The kinetics of this change was shown to vary monotonically with the potassium ion efflux across the membrane estimated from the integral over time of the potassium current divided by the Faraday, and to be independent of both the external sodium ion concentration and the presence or absence of membrane series resistance compensation.

The experimental part of this portion of the project was done at the Marine Biological Laboratory in Woods Hole, Mass., and the analytical part was done at Bethesda, making use of the Laboratory of Biophysics' new PDP-11 digital computer and DCRT's PDP-10 time sharing digital computer system.

2) Interaction of lanthanides with $Na^+$ and $K^+$ activation in squid axons. Giant axons from the squid, Loligo pealei, were exposed to artificial sea water solutions in which the normal calcium ions were replaced with a variety of rare earth metal ions (Lanthanum, Europium, Samarium and Yterbium). The effect of these lanthanides on the sodium and potassium membrane conductance channels was studied by means of the voltage clamp technique. Axons were voltage clamped by a method described previously and modified as follows: (1) the command potentials to the voltage clamp were generated by a computer (General Automation, SPC-12) through a D/A converter; (2) the membrane currents, converted to voltages by a current-voltage transducer, were scanned by a fast A/D converter (DataLab 710) and the digitized data was then recorded on 9-track digital magnetic tape; (3) the digital data was analyzed making use of large batch-processing digital computers at the Woods Hole Oceanographic Institute and at the State University of New York at Binghamton.

Major Findings:

1) Modification of ionic flow across neural membranes through ion concentration changes in periaxonal and perineuronal spaces resulting from membrane activity. In voltage clamping squid giant axons, current flows outward through the potassium conductance, $g_K$, upon membrane depolarization. It was assumed that during outward potassium current flow, potassium ions accumulated in a periaxonal space bounded by the membrane and an external diffusion barrier. A model system was used to describe this accumulation as a continuous function of the membrane currents. This model was fit to the voltage clamp data using the PDP-11 digital computer, and a least squares curve fit program. On this basis, the mean periaxonal space thickness and the permeability of the external barrier to $K^+$ were found to be 357Å and $3.21 \times 10^{-4}$ cm/sec, respectively. In hyperosmotic sea water, the value of the space thickness increased significantly even though the potassium currents were not changed significantly. Values of the resistance in series with the membrane were calculated from the values of the permeability of the external barrier and these values were shown to be equivalent to series resistance values determined by current clamp

measurements. Membrane potassium ion conductances were determined as a function of time and voltage. When these were determined from data corrected for the potassium current reversal potential changes, larger maximal potassium conductances were obtained than were obtained using a constant reversal potential. These data were fit with the Hodgkin and Huxley nerve equations, using the MLAB least squares curve fitting routine on the DCRT PDP-10 digital computer. In addition, the potassium conductance turn-on with time at a variety of membrane potentials was shown to be slower when potassium conductance values were obtained using a variable reversal potential than when using a constant reversal potential.

Two full papers are in preparation and the following presentations were made:

Experimental Tests of a Model System Accounting for Potassium Accumulation in the Periaxonal Space by W. J. Adelman, Jr., Y. Palti and J. P. Senft at the Second Annual Meeting of the Society for Neuroscience, October 8-11, 1972, Houston, Texas.

Further Evidence for Potassium Concentration Change in the Periaxonal Space of the Squid Giant Axon by W. J. Adelman, Jr., and J. P. Senft, at the Society of General Physiologists, 26th Annual Meeting, September 6-9, 1972, Marine Biological Laboratory, Woods Hole, Mass.

The Effects of Temperature on Axon Membrane Ionic Conductance by Y. Palti, W. J. Adelman, J. P. Senft and N. Moran in Israel, 1972.

One invited lecture and one workshop by W. J. Adelman, Jr., by invitation of Dr. D. E. Goldman, Program Director of Lectures and Workshops of the Excitable Membrane Training Program, 1972, at the Marine Biological Laboratory, Woods Hole, entitled "Squid Dissection and Recording" and "Periaxonal Space", July, 1972.

2) Interaction of lanthanides with $Na^+$ and $K^+$ activation in squid axons. Lanthanide ions have many chemical properties in common with $Ca^{++}$; in particular, their crystal radii are similar. The interaction of the lanthanides with sodium and potassium membrane conductance channels was studied using the voltage clamp technique. Squid axon membrane currents were obtained in artificial sea water solutions containing zero $K^+$ and $Mg^{++}$, and 10 mM concentration of lanthanide ions substituted for the normal 10 mM $[Ca^{++}]$. Resting and action potentials were also recorded. All lanthanides gave a two rate constant decrease in resting potential (an initial reversible rapid fall, followed by a slow irreversible decline). During the rapid fall, the action potential amplitude remained nearly constant, but the duration increased. Action potential amplitude declined during the slower depolarizing phase. These changes were accompanied by an inhibition of $g_{Na}$ and $g_K$ as determined from voltage clamp analyses. In the initial phase, $g_K$ was reduced more than $g_{Na}$. The effectiveness of the lanthanides as conductance channel inhibitors was in the series: $Sm^{++} = Eu^{++} > Yb^{++} > La^{++}$. The kinetics of these ionic interactions as well as those of $Ca^{++}$ with $g_{Na}$ and $g_K$ have been analyzed in terms of the Hodgkin and Huxley parameters which describe these membrane conductances.

Presentations were made as follows:

Interaction of Lanthanides with $Na^+$ and $K^+$ Activation in Squid Axons, by M. Starzak, J. P. Senft and W. J. Adelman, Jr., at the Society of General Physiologists, 26th Annual Meeting, September 6-9, 1972, Marine Biological Laboratory, Woods Hole, Mass.

Interaction of Lanthanides with $Na^+$ and $K^+$ Activation in Squid Axons by M. Starzak, J. Senft and W. J. Adelman, Jr., at the Fall Meeting of the American Physiological Society, August 28-September 1, 1972, Pennsylvania State University.

Significance to Biomedical Research and the Program of the Institute:

1) The findings with respect to $K^+$ accumulation in periaxonal space have led to a general model of the phenomenon which may be applied to many neural systems, both peripheral and central. The significance of potassium accumulation reaches beyond the above described effects in axons. There have been a number of reviews recently which have marshalled the evidence for the transport of sodium and potassium ions across the axolemma and other living membranes as a function of the external potassium ion concentration. In the central nervous system natural repetitive activity of axons and neurons must result in $K^+$ accumulation. The resulting $K_s$ changes will affect the firing rates, integrative functions, transmitter release, etc., in these systems. For example, during hyperpolarization which follows a train of impulses the neuron membrane is hypersensitive to external potassium, and the $[K_s]$ effect may be considerably amplified under a variety of conditions. In addition, some widespread CNS phenomena such as spreading cortical depression and epliptiform cortical discharges have been explained on the basis of changes in $[K_s]$ in modifying the activity of both peripheral and central nervous systems has been reviewed and compared with our experimental and theoretical work in the squid giant axon (Adelman and Palti, 1972).

2) It is hoped that the lanthanide interaction with the ionic conductance channels in nerve membrane will provide detailed information as to both channel geometry and the role of divalent ions in neural membrane mechanisms.

Proposed Course of the Project:

1) Frankenhaeuser and Hodgkin have stated that, "one limitation of the theory put forward by Hodgkin and Huxley on the basis of voltage clamp records is that the equations do not predict a negative after-potential. It is now evident that this limitation arose because the analysis took no account of the polarization effect observed with large outward currents". We (Adelman and FitzHugh) plan to show that with $\bar{g}_K$, $\alpha_n$ and $\beta_n$ values obtained with

appropriate corrections made for $K_s$ accumulation this limitation can be partially overcome. On the basis of data outlined in this report, we plan: (1) to predict the exact form and shape of the action potential in the squid axon, (2) to predict the time course of repetitive firing in axons, (3) to account for the measured potassium ion accumulation associated with both single and repetitive nerve impulses.

2) Further work is planned to work out the role of lanthanides in the modification of membrane conductance parameters. This work will be performed in cooperation with Dr. Starzak of the State University of New York at Binghamton, and Dr. Senft of Juniata College.

Honors and Awards: None

Publications:

Adelman, W. J., Jr., and Palti, Y.: The role of periaxonal and perineuronal spaces in modifying ionic flow across neural membranes. In Bronner, F. and Kleinzeller, A. (Eds.): Current Topics in Membranes and Transport, New York, Academic Press, 1972, Vol. 3. pp. 199-235.

Serial No. NDS(I)-72 LB/OC 1967
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electrical Fluctuations of Excitable Membranes.

Previous Serial Number: Same

Principal Investigator: K.S. Cole, Ph.D.

Other Investigators: H.M. Fishman, Ph.D. and T.L. Hill, Ph.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

State University of New York at Albany, Albany, New York.

Laboratory of Molecular Biology, NIAMD, NIH.

University of California, Berkeley, Calif.

Man Years:

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.2 |
| Other: | 0.0 |

Project Description:

Objectives: Interpretation of the function of excitable mechanisms in terms of ionic permeabilities and the elucidation of the structure and mechanisms by which the permeabilities are controlled.

Methods Employed: The preliminary crude attempts to measure potential or current fluctuation spectra and auto-correlation functions of membranes and electrolytes were begun at the Laboratory of Biophysics and have been continued as a joint project at the State University of New York, Albany, (SUNY). The simple on-line spectral analyzer used for μ tips has been replaced by powerful sweep analyzers, tape recorders and auto-correlators under an NIH grant. The principal emphasis has been on obtaining the small patch of squid axon membrane needed for measureable noise which sucrose isolation has produced. Investigations of electrolyte model systems have been continued with aqueous electrolytes in the anisotropic configurations of μ tip, porous membranes and single pores in mica sheets and with non-aqueous electrolytes for uniform current densities.

Major Findings: The initial measurements on the squid axon agreed with results on frog node and lobster axon in showing a 1/f, or pink, excess noise and attributing it to potassium current fluctuations. Further squid experiments with increased precision and resolutions have shown a component of the form $1/(1+\omega^2\tau^2)$, where $\omega=2\pi f$, in addition to the predominant 1/f--as also noticed by Verveen, et al., for the node. The component is identified with potassium current by TEA sensitivity and the temperature coefficient of $\tau$ is as given by Hodgkin, et al. The values of $\tau$ agree closely with the Hodgkin-Huxley potassium time constant $\tau_n$ over a considerable range of membrane potential. But $\tau$ is less than that of the fluctuations predicted by the Hodgkin-Huxley potassium current formulation. By elimination 1/f noise and the equally mysterious leakage current seem closely connected.

Numerous and powerful theoretical investigations have not yet produced a satisfactory model for the potassium noise which will also agree with the bulk of other experimental evidence adequately summarized in the Hodgkin-Huxley equations. The work is reported separately by T.L. Hill under Project Serial No. NIAMDD-LMB-19.

Significance to Biomedical Research and the Program of the Institute: There seems little doubt but that the current or potential fluctuations are indications of nature of motion of individual K ions as they cross a membrane and so far this is the only direct observation of this fundamental process of ion permeability. It should be discouraging that, in the four decades since pink noise was first found, we have no indication of the mechanisms and no agreement on the facts for any single system whether conduction is by electrons, holes or ions. On the other hand, there are about a dozen groups working on theory and/or experiment for biological membranes. These groups are more cooperative than competitive and at least some of the credit for this situation and whatever may come out of it belongs to the Laboratory of Biophysics.

Proposed Course of the Project: The experimental equipment and procedures have been improved to give better potassium data in shorter experiments and hopefully also a measure of the sodium current fluctuations. The implications and analyses of the data can only proceed slowly in the absence of a promising model and because of the increased teaching load and lack of assistant and student support for the principal investigator.

Honors and Awards:

The book "Perspectives in Membrane Biophysics: A Tribute to Kenneth S. Cole" was edited by Daniel Agin and published in January, 1973. Sixteen articles were contributed by twenty-one authors--of whom eight are present or past staff members and three were guests of the Laboratory of Biophysics, seven others have also been associated with the Laboratory and three are not known to have had any other than personal connections.

The Membrane Group of the Biophysical Society established a "Kenneth S. Cole Award" to consist of a newly-created medal and an honorarium for "Contributions to the knowledge of membranes". The first Award was presented to D.E. Goldman by Dr. Cole in February, 1967.

Publications:

Fishman, H.M.: Relaxation spectra of potassium channel noise from squid axon membranes. Proc. Natl. Acad. Sci. USA 70: 876-879, 1973.

Serial No. NDS(I)-73 LB/OC 2023
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Vitamin D Induced Changes in Renal Membrane ATPase System.

Previous Serial Number: None

Principal Investigator: A.E. Shamoo, Ph.D.

Other Investigators: F. Bronner, Ph.D., Marie Cassidy, Ph.D. and P.J. Ouseph, Ph.D.

Cooperating Units: Department of Oral Biology, School of Dental Medicine, University of Connecticut, Storrs, Connecticut.

Department of Physiology, George Washington University, School of Medicine, Washington, D.C.

Department of Physics, University of Louisville, Louisville, Kentucky.

Man Years:

| | |
|---|---|
| Total: | 0.3 |
| Professional: | 0.3 |
| Others: | 0.0 |

Project Description:

Objectives: Vitamin D-deficiency causes a decrease in the intestinal absorption of calcium, and a correlated decrease in intestinal Ca-dependent ATPase. Therefore, it was of interest to investigate renal membrane ATPase and renal reabsorption of Ca in Vitamin D deficient animals.

It was found $Fe^{2+}$, not $Fe^{3+}$, inhibits specifically the Mg-dependent ATPase. Thus to take advantage of the specific interaction between $Fe^{2+}$ and Na+K-ATPase $Fe^{2+}$ was used as a molecular probe to study the enzymatic reaction of Na+K-ATPase. $Fe^{2+}$ is known to be a Mossbauer element. Mossbauer spectrum of $Fe^{2+}$ in the presence of the enzyme and the different cations were used to elucidate the molecular mechanism of Na+K-ATPase.

Methods Employed: After the attainment of Vitamin D-deficiency in rats (by the method of Hurwitz, et al.) the rat kidneys were removed. Microsomal (100,000 g) fractions were prepared by differential centrifugation. Microsomes from turtle bladder were obtained in the usual fashion. Then the Mossbauer spectrum of $Fe^{2+}$ interaction with the enzyme and the cations were studied.

Major Findings: In Vitamin D deficient rat kidneys, both Ca and Mg dependent ATPase levels decreased by over 35% and 50%, respectively, from levels found in normal rats (D-repleted). After Vitamin D treatment, the levels of activity were restored to nearly normal levels (+D) (i.e., pre-deficiency levels). After Vitamin D-treatment, the Mg-dependent, Na+K stimulated ATPase increases by 30% over the control level (+D). It is inferred that in D-deficient rats, the normal Ca-absorption in the kidney is due to the coupling between Na-active transport and Ca-transport. Thus, the increase in renal Na+K-ATPase in D-deficient states might compensate for the decrease in renal Ca-ATPase.

Ouabain increases the quadrupole splitting Mossbauer spectrum of $Fe^{2+}$ in the presence Mg, Na and K. The increase in quadrupole splitting indicates that ouabain keeps two charge centers further apart. This may explain the inhibitory action of ouabain on Na+K-ATPase.

Significance to Biomedical Research and the Program of the Institute: This project points out the interrelation between a vitamin and an enzyme system fundamental to the functioning of all neural systems.

Proposed Course of the Project: This project is now complete.

Honors and Awards: None

Publications: None

# Annual Report
July 1, 1972 through June 30, 1973
Laboratory of Experimental Neurology, IR
National Institute of Neurological Diseases and Stroke

William F. Caveness, M. D.

## General Statement

This is a small laboratory with five permanent and one temporary position. Its productivity is dependent upon the conceptual and technical competence of each of its staff, the effective contract support for processing of anatomical specimen and supply of newborn monkeys, and the callobration of scientists at Harvard University, and the Armed Forces Institute of Pathology.

This activity is a continuation of a primate laboratory established at Columbia University in 1958 as an adjunct to the Seizure Clinic for Children, Columbia Presbyterian Medical Center, both under the direction of one of us. Its initial purpose was to better understand clinical seizure patterns in infants and young children by studying the evolving seizure patterns in the developing brain of the monkey. The first eight years of this investigation is summarized in Ontogeny of Focal Seizures, a chapter in Basic Mechanisms of the Epilepsies, Little Brown and Co., 1969. In 1961, in collaboration with Brookhaven National Laboratories, a series of studies were begun on the delayed effects of x-irradiation on the cerebral cortex of the Macaca mulatta. This was prompted by the clinical observations on the wards of the Neurological Institute of New York of delayed spinal cord necrosis that followed in a matter of months the unintentional exposure of the cord during irradiation therapy for neoplasms of the nasopharynx, lung or mediastival structures. The results of the first six years of these investigations are set forth in Pathogenesis of X-irradiation Effects in the Monkey Cerebral Cortex, Brain Research, 1968, 7: 1, Special Issue.

The official establishment of the Laboratory within the Intramural Program, NINDS, NIH, was completed on April 4, 1969 and the physical transfer of the Laboratory records, equipment and supplies from New York to Bethesda took place on March 21, 1970. Essential to the continuity was the transfer of our Biological Laboratory Technician who had been with the laboratory for six years.

## Current Scientific Effort:

### 1) Ontogeny of Focal Seizures in the Monkey.

Earlier electrographic and clinical findings, indicative of the dependence of the seizure pattern on regional organization in the central nervous system, led us to the postulate that while the cerebral cortex in the newborn is able to support focal paroxysmal activity, it is dependent upon the subcortical somatotopically linked nuclear masses and circuits for the full development of focal abnormality, its propagation to other parts of the cortex and to a common final pathway that results in the clinical seizure. In the 24 month old (pubescent) monkey, on the other hand, the propagation is under the control of cortical circuitry.

To test this concept, two experimental procedures were carried out just prior to penicillin induced focal activity in the cortical motor face area. By undercutting the site of activation, the propagation was blocked in the newborn and unimpeded at 24 months of age. Interrupting the intracortical connections by circumsecting the activation site had negligible effect in the newborn, but blocked the propagation at 24 months. These findings support the concept that the effective neuronal organization for the propagation of focal paroxysmal activity shifts from subcortical to cortical with maturation.

During the past year, the anatomical substrate for the seizure activity in the cortex has been extensively investigated with particular attention to the developing fiber systems, as delineated by Nauta techniques, and the developing pyramidal and stellate cells as demonstrated in Golgi preparations. At birth, cortico-cortical connections are poorly developed, and cortico-subcortical circuits are well demarkated. In contrast, cortico-cortical connections are prominent at 24 months of age. This increase in cortical connectivity is reflected in the postnatal architectonic development of layers II and III, the loci of the most dramatic events of the postnatal development.

Regional Blood Flow Studies: A logical extension of the investigation of the propagation of focal seizures in the newborn monkey is a search for the subcortical circuits and nuclear masses that are involved in the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. For us, it seemed wise to determine the natural course of events by noting the regional increase in blood flow, using the $^{14}C$-labeled antipyrine method developed by Sokoloff et al, at sequential intervals after the induction of the seizure. A preliminary survey, in twelve monkeys, has indicated the appropriatness of this method. Two advantages in exploring subcortical mechanisms in the newborn will be exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. Once the natural progression is delineated then one may appropriately investigate individual components with depth recording or interruption of activity by a cryoprobe.

Significance: The results from these investigations should improve the understanding and management of age dependent seizure patterns in humans.

## 2) Delayed Effects of X-irradiation on the CNS of the Monkey.

The established model utilizes a single exposure of 3,500 rads to the right visual cortex in the yound adult Macaca mulatta. This permits a precise measurement of functional changes as expressed in the visual evoked response. Sequential structural studies have shown ultrastructure evidence for cellular changes in carbohydrate and protein metabolism within days of exposure, alteration in the dendritic plexus within four to twelve weeks, and the proliferative and degenerative changes of radionecrosis in the exposed area four to five months following irradiation. Long-term functional alterations have included, first unilateral and then bilateral depression of evoked response and behavioral blindness. Long-term structural changes have included periodic brain swelling and retrograde transsynaptic degeneration within the visual system.

Three series of animals have been employed in the past two years to determine the mechanisms operating in the long-term phenomena. The first consisted of twelve monkeys with implanted cerebral spinal fluid catheter-reservoir systems, permitting sequential monitoring of the CSF pressure following irradiation. These, with two controls, were prepared in August 1971. In eight animals there was relatively abrupt brain swelling, 18 to 35 weeks after irradiation, reflected by an increase in CSF pressure, 3-4 times baseline values, with absence of visual evoked response and behavioral blindness in four, and marked VER depression in four. Three had papilledema. Seven of these, sacrificed between 20 and 36 weeks, one hour after i.v. Evans Blue, 2cc/kilo, showed proliferative and degenerative changes in the right occiput, deeply stained with dye. The swelling extended, beyond the irradiated and stained area, throughout the right hemisphere with distortion in brain structures. One sacrificed at 52 weeks, after resolution of the increased pressure and return of VER, had a fibrotic, non stained occipital lesion and no brain swelling. The remaining four animals showed variations in CSF pressure, seldom exceeding twice baseline value, over 52 weeks; moderate reduction in the visual evoked response; and moderately swollen brains, with Evans Blue staining of the irradiation site, at sacrifice.

The significant feature in the delayed brain swelling was the breakdown of the blood brain barrier that accompanied the delayed radiation lesion, with loss of serum fluid into the brain parenchyma. The impaired function was related to local destruction in the right visual cortex and widespread ipsilateral vasogenic edema and contralateral compression of the right and left visual pathways, respectively.

A second series of 18 monkeys was prepared with a subarachnoid catheter-reservoir system and irradiated in August, 1972. To investigate the alteration in cerebral blood flow that accompanies the evolution and resolution of vasogenic edema the regional blood flow is being determined at five discrete points on the CSF pressure curve: 1) baseline, 2) ascending, 3) peak, 4) descending, 5) complete resolution, utilizing the $C^{14}$ antipyrine technique.

A third series of four monkeys had the right visual cortex surgically ablated in

March 1972 without exposure to x-irradiation. Their course will be followed by visual evoked responses and they will be sacrificed at one, two, three, and four years respectively. The first was sacrificed, after one year, in March 1973. This series should demonstrate the effect of the loss of the right visual cortex, without brain swelling, on the transsynaptic degeneration in the visual system. By utilizing the findings from these three series, there should be a better understanding of the long-range effect of x-irradiation in the model under study.

Note: The sequence of structural changes, proceding from intracellular to local tissue, to remote and generalized effects, with remissions and exacerbations, strongly suggest isoimmune reactions and/or the activation of an infectious agent (slow and latent viruses). To test whether such a factor is present and transmissible, two newborn monkeys have received cerebral injections of material from the lesions in monkeys with post irradiation necrosis. One newborn control received a similar injection of material from a non-irradiated monkey. These will be observed for 24 months.

Planning 1973: Exploration of delayed effects following 6,000 rads to the whole head, in divided doses, over 28 days, a standardized therapeutic practice being employed by the Cancer Institute in controlled human studies.

Significance: The results from these investigations should have meaning and practical prognostic significance in the increasing human exposure to radiation in therapeutic efforts, the use of nuclear energy, and future space exploration.

### 3) Vietnam Registry.

A registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the Navy and was implemented with the cooperation of the Surgeons General of the Army and Air Force. The purpose was to insure uniformity of data collection and to identify cases for present and future studies. The yield from 1967 to 1970 was 2,043 entries that included 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 22.2 years. Of the head injuries, 90% were the result of explosively propelled missiles, 10% the result of vehicular accidents, falls, or blunt objects. In 85% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. 60% of these were accompanied by immediate loss in consciousness, by history. 30% were in coma, responding only to pain at time of examination, that was generally within six hours of injury. This provides an opportunity to group selected cases by regions of focal damage, with and without evidence of alteration in consciousness. A formal report of the Registry content and an estimate of its usefulness was transmitted to the Surgeons General, copy to the Director, Intramural Research, in November, 1972.

Significance: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma.

Serial No. NDS(I)-69 LEN/OC 1693
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Functional and Structural Alterations Following X-irradiation of the Cerebral Cortex in the Monkey

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M.D.

Other Investigators: Akira Tanaka, M.D.
Kenneth H. Hess
Thomas L. Kemper, M. D.
Lorenz E. Zimmerman, M.D.
Mark O. M. Tso, M. D.

Cooperating Units: Harvard University Medical School
Armed Forces Institute of Pathology

Man Years

| | |
|---|---|
| Total: | 2.3 |
| Professional: | 1.1 |
| Other: | 1.2 |

Project Description

Objectives: To determine the pathogenesis of early and late effects of irradiation on the central nervous system.

Methods Employed: The right visual cortex, in the young adult macaca mulatta, is exposed to 3,500 rads of x-irradiation in a single dose. Functional changes are monitored by evoked electroencephalographic responses from photic stimulation of the retina prior to and at weekly intervals, following irradiation. The stimuli are programmed to provide single light flashes that may be averaged and a series of frequencies that range from three to thirty flashes per second in ten second epochs. The evoked response from the irradiated and non-irradiated visual cortex is led through a two-channel Grey-Walter, low frequency analyzer that provides histograms for the abundance of activity, or amplitude, for ten seconds at each of the frequencies employed. Thus one can compare directly the amplitude of the evoked response from the irradiated cortex with that from the homologous non-irradiated cortex. In addition, the frequency analyzer detects delta activity, one to three cycles per second, when it appears as a reaction to injury. The averaged evoked response of a series of thirty-two flashes at one second intervals has provided a sensitive indicator of alteration in wave form that is especially useful when the differences between the irradiated and non-irradiated side are less pronounced.

Sequential anatomical examinations have been carried out by sacrificing three animals at 72 hours, 1, 4, 12, 20, 28, 44 and 144 weeks for routine, Golgi-Cox and electron microscopic preparations. Six animals have served as controls.

Major Findings: In the first ten months the functional changes included an initial and secondary unilateral depression in the photic evoked response in the right visual cortex. There was ultrastructure evidence for changes in carbohydrate and protein metabolism within three days of exposure, alteration in the dendritic plexus within 4 to 12 weeks, and radionecrosis, in the exposed area, within 4 to 5 months following irradiation. (Special Issue of Brain Res. 7: 1-120, 1968) In nine additional preparations, long-term functional alterations, up to 36 months, have included bilateral depression of evoked response and behavioral blindness. The long-term structural changes have included periodic brain swelling, and retrograde transsynaptic degeneration within the visual system.

During this fiscal year, three new experimental procedures have been exploited to define the mechanisms operating in the long-term phenomena.

The first consisted of fifteen monkeys with implanted cerebral spinal fluid catheter-reservoir systems that permitted sequential monitoring of the CSF pressure following irradiation. The catheter, .065 cm O.D., .030 cm I.D., made of medical grade silicone rubber, was inserted in the subarachnoid space at L 2-3. The cephalad projection was 18-22 cm that approached or entered the cisterna magna. The caudal end was connected to a modified side inlet reservoir, 1.5 cm in diameter, which was then secured beneath the skin. For the measurement of the CSF pressure, the animal was placed in a prone position with the head fixed, the limbs comfortably restrained, the eyes covered, and the environment quiet. With sterile technique a 22 gauge needle was passed through the skin into the reservoir. The needle was connected to a closed manometric system that measured the pressure above the level of the heart. Pressure readings were carried out over a thirty minute period to obviate effects of agitation. Six weeks (4 to 8) after the implantation of the catheter-reservoir system, the right visual cortex was irradiated in the usual manner in thirteen of the animals; two were maintained as controls.

Eight of the irradiated animals showed an abrupt elevation in pressure, accompanied by papilledema and/or bilateral obliteration of the visual evoked responses, between four and five months following exposure. These were sacrificed and their orbs and brains are being subjected to definitive histological examination. The remaining five animals showed less abrupt and less extensive elevation in pressure, followed by partial recovery. Their observation was terminated after 12 months. It is expected that this series will demonstrate the effect of brain swelling and papilledema on the degenerative changes within the visual system.

The second consisted of four monkeys with the right visual cortex surgically ablated, without x-irradiation. After exposure of the right occipital lobe, the parieto-occipital fissure was separated with the aid of a dissecting microscope and a glass pipette that was used as a cutting edge as well as for suction. The separation of the lateral aspect was extended up over the cortex to the medial aspect, with coagulation of vessels as indicated. The ablation was completed with the pipette extending the separation ventrally until the tentorium cerebelli was reached. The separated brain was removed manually. Subpial suction of the cortex along the edge of the intact portion of the brain eliminated the associated visual cortex completely. The course of these four animals will be followed by visual evoked responses and they will be sacrificed at one, two, three, and four years, respectively. The first of these was sacrificed in March 1973. This series should demonstrate the effect of the loss of the right visual cortex, without brain swelling, on the transsynaptic degeneration in the visual system. By utilizing the findings from these two series, there should be a better understanding of the long-range effect of x-irradiation in the model under study.

The sequence of structural changes, proceding from intracellular to local tissue, to remote and generalized effects, with remissions and exacerbations, strongly suggest isoimmune reactions and/or the activation of an infectious agent (slow and latent viruses). To test whether such a factor is present and transmissible, two newborn monkeys received cerebral injections of material from the lesions in monkeys with post irradiation necrosis. One newborn control received a similar injection of material from a non-irradiated monkey. This third procedure was completed in April 1973.

Significance to Bio-Medical Research and the Program of the Institute: The results from these investigations should have meaning and practical prognostic significance in the increasing human exposure to radiation in therapeutic efforts, the use of nuclear energy, and space exploration.

Proposed Course: Completion of the above plus the following. We will utilize the same parameters of observation following exposure of the brain to x-irradiation according to current therapeutic practice, e.g., 6000 rads to the whole head, in divided doses, over 28 days. This is in the planning phase.

Honors and Awards: None

Publications:

Caveness, W. F., Kato, M., Tanaka, A., Hess, K. H., Tso, M.O.M. and Zimmerman, L. E.: Delayed brain swelling after x-irradiation of the visual cortex in the Macaca mulatta. In: Brock, M., and Dietz, H. (Eds.): Intracranial Pressure. Springer-Verlag, Berlin, Heidelberg, New York, 1972

Hess, K. H., and Kato, M.: Comparison of techniques for analyzing visual evoked responses. Electroencephalogr. Clin. Neurophysiol. 34: 1973

Serial No. NDS(I)-69 LEN/OC 1694
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ontogeny of Focal Seizures

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M. D.

Other Investigators: Hideo Ueno, M.D.
Akira Tanaka, M.D.
Kenneth H. Hess
Thomas L. Kemper, M.D.

Cooperating Units: Harvard University Medical School

Man Years

| | |
|---|---|
| Total: | 2.0 |
| Professional: | .8 |
| Other: | 1.2 |

Project Description

Objectives: To better understand the evolving seizure pattern in the developing brain.

Methods Employed: Focal seizures are induced by injecting 25,000 units of penicillin in 0.025 cc of aqueous solution into the hand-face area of the motor cortex of the macaca mulatta at successive age levels from birth to young adulthood.

The onset and elaboration of the attack pattern is simultaneously recorded by electrocorticography, electromyography, and clinical observation.

The cerebra of the experimental, and a control, series of animals are examined for the level of development in cells, neuropil and myelin with attention to the motor cortex and subcortical tracts that are deemed relevant.

Major Findings: A significant feature of this investigation, in the macaca mulatta, has been the apparent dependency of the immature motor cortex on thalamic and other subcortical structures in the development and propagation of the paroxysmal discharge.

To test this concept, the cortical-subcortical pathways beneath the face area in the precentral gyrus were sectioned in eight newborn monkeys. The undercut cortex was then injected with 25,000 units of penicillin. The paroxysmal activity recorded from an array of cortical electrodes developed within the undercut area and, except for limited and transient expression, did not spread beyond this region, suggesting a marked dependency upon the subcortical circuitry for propagation of the focal abnormality. Another procedure, in eight newborn animals, involved a subpial section of the cortex on four sides that separated the face area from the remainder of the precentral gyrus and the frontal cortex anterior to the arcuate sulcus. When penicillin was injected into the circumsected cortex the paroxysmal activity developed and spread beyond the cortical sections in the superior and inferior motor strip and to other regions of the cortex with concomitant clinical expression of the seizure, suggesting a limited dependence upon the continuity of the cortex for propagation of the abnormal activity.

Both procedures were carried out in four monkeys at 24 months of age. The results, in contrast to the findings in the newborn, indicate far greater dependence on the cortex for the development and propagation of the paroxysmal activity.

During the past year, the anatomical substrate for the seizure activity in the cortex has been extensively investigated with particular attention to the developing fiber systems, as delineated by Nauta techniques, and the developing pyramidal and stellate cells as demonstrated in Golgi preparations.

At birth, cortico-cortical connections are poorly developed, and cortico-subcortical circuits provide the major pathway for the propagation of cortical activity. In contrast, cortico-cortical connections are prominent at 24 months of age. This increase in cortical connectivity is reflected in the postnatal architectonic development of layers II and III, the loci of the most dramatic events of the postnatal development.

Significance to Bio-Medical Research and the Program of the Institute: The results from this investigation should improve the understanding and management of the age dependent seizure patterns in humans.

Proposed Course: Continuation of the study of the relative significance of cortex and subcortical structures at the successive age levels in the development and propagation of the seizure discharge. See Project Number 1897.

Honors and Awards: None

Publications:

Caveness, W. F., Echlin, F. A., Kemper, T.L., and Kato, M.: The propagation of focal paroxysmal activity at birth and at 24 months of age in the macaca mulatta. Brain - In press

Kemper, T.L., Caveness, W. F., and Yakovlev, P. I.: The neuronographic and metric study of the dendritic arbors of neurons in the motor cortex of macaca mulatta at birth and at 24 months of age. Brain - In press

Serial No. NDS(I)-71 LEN/OC 1897
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Regional Blood Flow Studies

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M. D.

Other Investigators: Akira Tanaka, M.D.
Hideo Ueno, M.D.
Kenneth H. Hess
Louis Sokoloff, M.D.

Cooperating Units: Laboratory of Cerebral Metabolism, NIMH

Man Years

| | |
|---|---|
| Total: | 2.0 |
| Professional: | .8 |
| Other: | 1.2 |

Project Description

See 1693. To investigate the alteration in cerebral blood flow that accompanies the evolution and resolution of vasogenic edema that follows exposure to the cerebral cortex to ionizing irradiation, a series of 18 monkeys was prepared with a spinal subarachnoid catheter-reservoir system and irradiated in August, 1972. Utilizing the $^{14}C$ antipyrine technique of Sokoloff, Revich and others, the regional blood flow is being determined at five discrete points on the CSF pressure curve; 1) baseline, 2) ascending, 3) peak, 4) descending, 5) complete resolution. A correlation of regional blood flow with the structural changes in the brain at each period will be established.

See 1694. A logical extension of the investigation of the propagation of focal seizures in the newborn monkey is a search for the subcortical circuits and nuclear masses that are involved in the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. In lieu of duplicating their efforts, which are limited by the position of the probes, it seemed wise to determine the natural course of events by the use of blood flow techniques, using the $^{14}C$ antipyrine method at sequential intervals after the induction of the seizure. A preliminary survey has indicated the appropriateness of this method. Two advantages in exploring subcortical mechanisms in the newborn will be exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. Once the natural progression is delineated, then one may appropriately investigate individual components with depth recording or interruption of activity by a cryoprobe.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LEN/OC 1898
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Activation of Unilateral Visual Pathways

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M.D.

Other Investigators: Louis Sokoloff, M. D.
Charles Kennedy, M.D.
Motohiro Kato, M.D.
Hideo Ueno, M.D.
Kenneth H. Hess

Cooperating Units: Laboratory of Cerebral Metabolism, NIMH

Man Years

| | |
|---|---|
| Total: | 1.0 |
| Professional: | 0.5 |
| Other: | 0.5 |

Project Description

Objectives: To delineate the alteration in blood flow within the various components of the visual system after photic stimulation.

Methods Employed: This study has utilized three split brain preparations, young adult macoca mulatta with section of corpus callosum and optic chiasm, that had been observed as to their photic evoked response over a period of 12 months. The monthly routine included unilateral, and bilateral retinal stimulation. These and three control animals were subjected to $^{14}C$ antipyrine regional blood flow studies following stimulation of the right eye for three minutes. Three additional control animals received $^{14}C$ antipyrine studies without visual stimulation.

Major Findings: The frozen heads were sectioned for confirmation of the surgical procedures and autoradiography.

There were small anterior and posterior portions of the corpus callosum remaining in all three of the split brain preparations, and remnants of the optic chiasm in two, that detracted from the adequacy of the preparations. Nevertheless, the autoradiograms showed that the ratio of cerebral blood flow between the two sides showed a greater flow in the main visual pathway, except for optic nerve, optic radiation and superior colliculus, on the side that received the photic stimulation. Further this increased cerebral blood flow correlated well with the visual evoked response that was predominantly on the side of the photic stimulation. The controls showed no significant difference between the two sides.

Significance to Bio-Medical Research and the Program of the Institute: Fresh insight into the relation of neurological function to alteration in blood flow, as regards the visual system. The split brain preparations afforded an opportunity to compare the two hemispheres after unilateral stimulation.

Proposed Course: Completed

Honors and Awards: None

Publications: In preparation

Serial No. NDS(I)-72 LEN/OC 1952
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Vietnam Registry, Analysis of Yield

Previous Serial Number: None

Principal Investigator: William F. Caveness, M. D.

Other Investigators: Berkley L. Rish, CDR (MC) USN

Cooperating Units: Office of the Surgeon General, USN
Office of the Surgeon General, USA

Man Years

| | |
|---|---|
| Total: | 1.0 |
| Professional: | .5 |
| Other: | .5 |

Project Description

Objectives: To insure uniformity of data collection in combat head injuries and to identify cases for present and future studies.

Methods Employed: A Registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the Navy and was implemented with the cooperation of the Surgeons General of the Army and Air Force. The yield from field surgeons, 1967-1970, was 2,043 entries.

Major Findings: The Registry includes 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 22.2 years. Of the head injuries, 90% were the result of explosively propelled missiles, 10% the result of vehicular accidents, falls, or blunt objects. In 85% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. 60% of these were accompanied by immediate loss in consciousness, by history. 30% were in coma, responding only to pain at time of examination, that was generally within six hours of injury. This provides an opportunity to group selected cases by regions of focal damage, with and without evidence of alteration in consciousness.

Significance to Bio-Medical Research and the Program of the Institute: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma.

Proposed Course: The evaluation of these data is being continued with particular attention to wound characteristics and the events during the acute phase of injury.

Honors and Awards: None

Publications:

Caveness, W. F., and Rish, B. L.: Structural and functional derangement in head injuries from Vietnam. An exhibit presented at the following meetings: American Association of Neurological Surgeons, April, 1972, American Neurological Association, June, 1972, Congress of Neurological Surgeons, October, 1972, and Association of Military Surgeons of the United States, December, 1972.

Caveness, W. F., Hayes, G. J., and Kempe, L.G.: Combat head injuries with and without helmet "protection". Trans. Amer. Neurol. Ass., 1972

Rish, B. L., and Caveness, W. F.: Relation of prophylactic medication to the occurrence of early seizures following craniocerbral trauma. J. Neurosurg. 38: 155-158, 1973.

ANNUAL REPORT
July 1, 1972 through June 30, 1973
Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological Diseases and Stroke

Donald B. Tower, Chief

Two administrative developments are noteworthy. Early in CY 1973 a new section was added to the Laboratory when the Section on Neural Development and Regeneration was transferred from the Laboratory of Neuropathology and Neuroanatomical Sciences (LNNS), IR, NINDS, to the Laboratory of Neurochemistry (LNC). There has been active collaboration between the Section and LNC for a number of years, so that to a considerable extent the administrative shift recognizes the existing community of interest. As a result of the shift, LNC has acquired additional personnel (4 professional, 1 subprofessional, 1 guest worker and 1 part-time secretary) and space (located in Bldg. 9), as well as two research programs concerned with the trophic factors influencing the symbiosis between nerve and muscle and with the problems involved in functionally useful central axonal regeneration. For the FY 1973 report these programs are included with those of LNNS and will not be duplicated here. Suffice it to say that we consider this a most welcome move that can only strengthen the LNC and provide fresh viewpoints and stimulus to all of our programs.

On May 1, 1973, the Chief of the LNC assumed new duties as Acting Director, NINDS, and turned over direction of LNC to the Head of the Section on Enzyme Chemistry. The latter constitutes a recognition of the scientific stature and administrative capabilities of the new Acting Chief of the LNC. The Laboratory is in good hands and I have every confidence that it will continue to pursue a vigorous and fruitful program.

Scientifically the year has been outstanding. The attached individual reports attest in detail the significant progress achieved by each of the LNC Sections. Here it is sufficient to record the principal highlights. In the Section on Enzyme Chemistry the staff has achieved for the first time definitive solubilization of the Mg-dependent, Na-K-activated ATPase---a major accomplishment that provides a stable preparation suitable for chromatographic, electrophoretic, structural and kinetic studies. Estimations of subunit molecular weights and fluorescent probe studies are already under way. Combinations of derived peptide ionophores with black-lipid bilayer membranes are being evaluated. And investigations of rapid reaction kinetics by stop-flow techniques have been initiated. Clearly this program appears to be on the threshold of major break-throughs in the elucidation of the enzymological mechanisms responsible for the transduction of cellular energy into cationic transport across the cell membrane.

In the Section on Physiology and Metabolism comparative studies on plasma membranes of a variety of cell types have provided several significant findings. With respect to the problem of production of additional membrane (as in cell division, pinocytosis, etc.), current studies by section personnel indicate that increases in the plasma membrane biomass do not necessarily involve an equivalent increase (synthesis) of its component molecules, since many of the latter appear to exist pre-formed (latent) for "structuralization" as required. With regard to the question of the "sidedness" of isolated plasma membranes comparisons of enzymatic activity obtained on intact cells with that obtained after cell disruption have demonstrated a definite pattern of "sidedness" for certain membrane-bound enzymes. For example, 5'-nucleotidase and a ouabain-insensitive ATPase are clearly external in location, whereas adenyl cyclase is clearly internally oriented. Thus, for the first time specific, detailed biochemical evidence can be adduced to support the physiological concepts of molecular asymmetry of plasma membranes. These observations are of fundamental importance and give promise of major advances in our knowledge of the functional organization of cellular membranes.

Finally studies in the Section on Amino Acids and Electrolytes have clearly established that astroglia (in tissue culture) possess a mechanism for the potassium-dependent, mediated inward transport of chloride that is a saturable process exhibiting Michaelis-Menten kinetics. Thus, the observations obtained previously for incubated cortical slices *in vitro* and perfused cortex *in vivo* have been substantiated and can be attributed to the astrocytes. The apparent K values for uptake of chloride as a function of external potassium concentration are very close in all three systems, and in the tissue culture cells, culture in chloride-free media appears to grossly impair the ability of the cells to maintain normal intracellular concentrations of potassium. The uptake of chloride by astroglia is competitively inhibited by acetazolamide (Diamox), an observation that may implicate the enzyme, carbonic anhydrase, as the mediator of chloride transport. These and other studies discussed in the accompanying reports clearly establish the astrocyte as a key element in the fluid and ionic economy of the central nervous system.

This year we have added a new dimension to our research capabilities by contracting for two research studies extramurally. As reported in detail in one of the accompanying reports, these contracts involve studies, respectively, on the intracranial disposition of antitumor drugs and on the failure of cerebral capillaries to reperfuse following temporary vascular occlusion. Both contract studies utilize techniques developed in this Laboratory and reflect ongoing program interests in the Section on Amino Acids and Electrolytes concerned with barrier phenomena, capillary permeability, fluid and electrolyte distribution and energy metabolism. Progress in both contracts has been excellent and warrants their renewal for a second year. Experience with this type of adjunct to in-house projects has been valuable and provides for greater versatility in the prosecution of certain phases of such projects.

The investigations highlighted briefly here reflect our continuing concentration on studies of neural membranes. Despite the very fundamental nature of much of the research, its relevance to many important clinical problems is immediately obvious. Thus, in the work on astroglia already discussed, there are particularly significant implications for epilepsy, stroke and cerebral edema in which selective involvements of astrocytes occur. Our studies continue to enjoy the benefits of fruitful collaboration with numerous intra- and extra-mural scientific colleagues and the benefits of the important contributions by the young men and women who comprise our group of research associates and visiting and guest workers. This latter small and select group is a vital asset, which we value very highly.

Over the past several years the Laboratory has experienced a number of vicissitudes. Both in its survival and in its solid research progress the strength of its programs and the dedication of its staff stand out as key factors. As the Laboratory enters upon what may prove to be yet another era, we are confident that it will successfully discharge its mission and will continue to provide even greater understanding of the neurochemical bases for the functions and the dysfunctions of the nervous system.

Serial No. NDS(1)-61 LNC/AAC 810
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Metabolism and Functions of Free and Protein-Bound Amino Acids in Neural Tissues.

Previous Serial Number: Same.

Principal Investigator: D. B. Tower, M.D., Ph.D.

Other Investigators: Oscar Young; Thomas H. Gill, M.D.; Milton Brightman, M.D.; Helen Hess, M.D.

Cooperating Units: Section on Neurocytology, LNNS, IR, NINDS; Laboratory of Vision Research, IR, NEI.

Man Years:

| | |
|---|---|
| Total: | 0.2 |
| Professional: | 0.2 |
| Other: | 0 |

Project Description:

Objectives: To investigate the metabolic interrelationships (and factors affecting them) of amino acids, especially of the glutamate and aspartate group, in the free and protein-bound pools of neural tissues, with emphasis on cerebral cortex studied *in vitro*.

Methods: In addition to standard procedures utilizing slices or homogenates of neural tissues incubated under usual *in vitro* conditions, specific procedures and assay methods are referred to or outlined in the appropriate section of "Major Findings" below.

Major Findings:

(A) Comparative Studies: Circumstances beyond our control have curtailed (perhaps permanently) these studies. The 1972 Bering Sea expedition of the R/V "Alpha Helix" failed to yield any beluga whales because of an unexpectedly late break-up of river and estuarian ice and the lack of any run of whales up river while the ship could be assigned to the project. Moreover, the Canadian government has imposed a ban on any further whaling by Canadian fisheries, so that our source for brains from the great whales is cut off. It was not possible to schedule a collecting trip in the fall of 1972. Portions of the cerebrum of a 14.0 m. male sperm whale obtained 21 h after harpooning were fixed in glutaraldehyde-$H_2O_2$, buffered with cacodylate and post-fixed with osmium tetroxide at the Nova Scotia whaling station during a brief visit in July 1972. The specimens were brought back for electron-microscopic examination by Drs. Brightman and Westergaard in the NINDS Section on Neurocytology, LNNS. Preliminary sections demonstrate generally intact, well-preserved mitochondria and generally intact myelin sheaths, with some examples of partial separation of lamellae. Moderate swelling of glia processes was evident. Cell membranes and membranes of rough endoplasmic reticulum were poorly preserved, but their locations were clearly discernible as "negative" images. Lysosomes and cell nuclei appeared to be relatively intact. Additional observations are in progress at low power electron microscopy to survey the general characteristics of the cortical neuropil and the cytology of the underlying white matter.

(B) Tissue Culture Studies: The principal studies are described in detail in the accompanying project report [NDS(1)-61 LNC/AAC 811]. As reference points for the electrolyte studies, procedures for determining dry weight, DNA, RNA and total protein have been developed. For convenience in the electrolyte studies, the astrocyte and neuroblastoma cell lines are cultured and incubated on small glass cover slips in small Petri dishes containing the appropriate culture or incubation media. For dry weight and DNA the cultured cells were scraped off the cover slips with a rubber "policeman" and the suspension of cells was transferred to a centrifuge tube and centrifuged to sediment the cells into a pellet. The pellet was resuspended in a known volume of buffered saline-glucose medium. DNA determinations by the procedure of Hess and Thalheimer (J. Neurochem. 12: 193, 1965) were kindly carried by Dr. Helen Hess (LVR, NEI). Dry weights were obtained by collecting known and equal volumes of the cell suspension and of the suspending medium on respective pre-weighed Millipore filter circles. The circles were dried overnight at 80°C (higher temperatures rendered the Millipore material much to friable for subsequent handling) and after cooling in a desiccator were reweighed to obtain the gross and net (gross-medium) dry weights. Total protein contents can also be obtained on the cell suspensions, but for routine use a procedure applicable to the cultures on the cover slips (without removal) is required in order to permit direct correlation with electrolyte analyses on such cover-slip cultures. For this purpose, cover slips with cultures still intact on their surfaces are placed in homogenizing tubes and homogenized (cover slip and all) in appropriate volumes of reagent solutions. The Folin-phenol procedure of Lowry et al. (J. biol. Chem. 193: 265, 1951) was used, with bovine serum albumin as standard. This procedure has proved very satisfactory and highly reproducible. For 35 determinations, the mean value (± S.D.) was 110 (± 9.9) μg per coverslip culture for the Shein hamster astrocyte cultures.

Significance: This project bears directly upon the fundamental problem of delineating some of the interrelationships between cerebral structure and metabolism, and between metabolism and functional activity, so that an eventual integration of these facets of cellular neurochemistry can be realized.

Proposed Course: To continue studies on tissue culture lines specifically oriented to evaluation of various metabolic parameters (anaerobic glycolysis) and enzymes (cholinesterase, glutamate decarboxylase, and α-glycerophosphate dehydrogenase) that are specific for one of the various cell types being cultured.

Honors and Awards:

Tower, D. B.: Invited to present the Plenary Doctrinary Lecture on "The Biochemistry of the Epileptogenic Brain " at the III Congreso Mexicano de Cirugia Neurologica, Acapulco, Mexico, March 19-24, 1973.

Publications:

1. Purpura, D. P., Penry, J. K., Tower, D. B., Woodbury, D. M., and Walter, R. D. (eds.) Experimental Models of Epilepsy. A Manual for the Laboratory Worker. New York, Raven Press, 1972.

2. Tower, D. B., and Young, O. M.: Interspecies correlations of cerebral cortical oxygen consumption, acetylcholinesterase activity and chloride content: Studies on the brains of the fin whale (Balaenoptera physalus) and the sperm whale (Physeter catodon). J. Neurochem. 20: 253-267, 1973.

Serial No. NDS(1)-61 LNC/AAC 811
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Reports
July 1, 1972 through June 30, 1973

**Project Title:** Electrolytes and Energy Metabolism in Cerebral Tissues.

**Previous Serial Number:** Same.

**Principal Investigators:** D. B. Tower, M.D., Ph.D.; T. H. Gill, M.D.

**Other Investigators:** O. Young; S. Goldman, Ph.D.

**Cooperating Units:** Dept. of Neurosurgery, Roswell Park Memorial Institute, Buffalo, N. Y.; Dept. of Neurology, Albert Einstein College of Medicine, Bronx, N. Y.

**Man Years:**

| | |
|---|---|
| Total: | 3.1 |
| Professional: | 2.8 |
| Other: | 0.3 |

**Project Description:**

**Objectives:** To study the *in vitro* metabolism of electrolytes and of energy-producing cycles and compounds thereof, primarily in incubated slices of cerebral cortex and subcortical white matter (or in equivalent whole-cell preparations, such as tissue culture) and where possible to extend and evaluate such studies *in vivo*.

**Methods:** In addition to established, standard methods for preparation and incubation of slices (or homogenates) of brain tissue or cultures of specific cell lines *in vitro*, with use of appropriate media and indicator solutes, specific procedures and applications are referred to or outlined in the appropriate sections of "Major Findings" below.

**Major Findings:**

(A) **Tissue Culture Studies:** Definitive evidence has now been obtained that the hamster astrocyte cell line originally isolated by Shein *et al*. (Brain Res. 19: 497, 1970) possesses a mechanism for the K-dependent mediated uptake of $^{36}$Cl by a saturable process that conforms to Michaelis-Menten kinetics. A suitable technique has been developed that permits counting of radioactivity, protein analyses or electrolyte analyses without the necessity of harvesting the cultured cells. The cells are cultured and incubated on small glass cover slips in small Petri dishes containing the appropriate culture or incubation media.

For isotope studies the cover slips are rinsed briefly in appropriate salines and counted in scintillation fluids by liquid scintillation spectrometer. The chemical analyses can be carried out after homogenizing the cultures (cover slip and all) in appropriate solutions and analyzing the resulting solutions for protein (by the Lowry procedure) or for electrolytes (by emission flame photometry for Na and K or by amperometric titration for Cl).

When the astrocyte cultures are incubated for 1-60 min at 37°C in bicarbonate-buffered saline-glucose media containing 140 mM Cl, the initial rate of uptake and total uptake of $^{36}$Cl are functions of the external K concentration over the range 5-54 mM. Net uptake of $^{36}$Cl is obtained by correcting for adherent and extracellular medium by adding [$^{3}$H] inulin to the incubation media. The uptake is temperature dependent and is probably energy dependent since it is completely inhibited by 2,4-dinitrophenol or fluoride. A Lineweaver-Burk plot of the reciprocal of the initial velocity of uptake of $^{36}$Cl as a function of the reciprocal of the external K concentration indicates a saturable process with an apparent $K_m$ of 38.5 mM (i.e., the concentration of external K promoting half-maximal uptake of $^{36}$Cl). This value falls in the range found for the K-dependent mediated uptake of $^{36}$Cl by cerebral cortical slices incubated *in vitro* ($K_m$ = 30.3 mM) and cerebral cortex perfused *in vivo* ($K_m$ = 47.6 mM) reported previously. None of these phenomena can be demonstrated for cultures of murine neuroblastoma cells (clone C-1300, subclone N-18; Seeds *et al.*: Proc. nat. Acad. Sci., U.S.A., 66: 160, 1970) incubated under identical conditions.

Moreover the uptake of $^{36}$Cl by cultured astrocytoma cells is inhibited by other anions, notably 5 or 10 mM bromide, iodide, thiocyanate or perchlorate, and by 5 or 10 mM acetazolamide (Diamox). Lineweaver-Burk plots of the uptake of $^{36}$Cl in the presence of 10 mM perchlorate or 10 mM Diamox as functions of external K concentrations indicate that the inhibition of $^{36}$Cl uptake by these compounds is competitive. We would have expected the inhibition by other anions to be competitive but it was surprising that the inhibition by Diamox is also competitive. Diamox is a specific inhibitor of carbonic anhydrase (the enzyme catalyzing the reversible reaction: $H_2CO_3 \rightleftharpoons CO_2 + H_2O$) and is known to inhibit chloride transport in all other systems examined (erythrocyte membrane; gastric mucosa; choroid plexus) but we are not aware that such detailed kinetics have been examined in these other systems. There are reports that chloride can combine directly with the carbonic anhydrase enzyme, so that our observations may reflect transport of $^{36}$Cl mediated by the enzyme itself. The technical problems of evaluating this possibility in whole cell preparations where intracellular pH and $CO_2$ cannot be measured or controlled are so formidable that we think it unlikely that any definitive results could be obtained. The alternative of studies with the pure enzyme is being evaluated.

One final observation indicates the importance of chloride to the electrolyte composition of the cultured astrocytes. Like the previous reports we found an intracellular K/Na ratio of 4.55 when the cells were cultured or incubated in chloride-rich media. In chloride-free media (substitution of isethionate for chloride) the K/Na ratio became 0.43. Thus in the absence of external chloride these astrocytes, which are known to be high K cells by both neurophysiological and neurochemical analyses, are unable to maintain a high intracellular K concentration.

(B) Contract Studies: In 1972 two contracts were negotiated with the Roswell Park Memorial Institute, Dept. of Neurosurgery, with Drs. Robert Bourke and Charles West as investigators. These contracts call for studies utilizing the bilateral cortical superfusion techniques developed for primate brain in this laboratory by Dr. Bourke. (1) One contract involves studies on the kinetics of entry and procedures for increasing entry and residence time of antitumor agents in the central nervous system. During this year such data for 5-fluoruracil (5-FU) have been obtained and will be published in the July 1973 issue of Cancer Research. This drug was chosen as a model drug that is not bound by plasma proteins, that is not appreciably metabolized, and that has relatively ready access to the CNS. Comparable studies on methotrexate are currently in progress, and the effects of small doses of X-irradiation to the head or of variations of arterial $pCO_2$ on CNS permeability to these drugs. (2) The other contract involves studies of whether or not the "no-reflow" phenomenon following experimentally-induced temporary cerebrovascular occlusion (stroke) occurs in the primate cerebral cortex in vivo. The studies utilize the West procedure for producing experimental stroke and $[^{14}C]$-or $[^{3}H]$ antipyrine to evaluate capillary flow and permeability across the cortex between the vasculature and the superfusion fluid. It is clear that the "no-reflow" phenomenon does indeed occur even after brief (15-30 min occlusions); however, it was found that the capillary flow and permability is exquisitely sensitive to local $pCO_2$ (critical range 30-40 mm Hg) so that this factor must be rigorously controlled to provide intelligible, reproducible data. Studies are currently in progress to extend the observations to blood and perfusate pH, $pO_2$ and $pCO_2$ with the use of three simultaneously recording mass spectrometers added to the experimental set-up.

Significance: Energy-yielding metabolism is the basic factor underlying neuronal function and activity, and electrolyte metabolism (which clearly depends upon it) provides a fundamental link between cellular chemistry and the functional activity of impulse conduction. The understanding of factors involved is essential for the elucidation of both normal functioning of neural tissues as well as deranged functions of hyperactivity states.

Proposed Course: To continue investigations along the lines indicated. In the tissue culture studies we will attempt to extend the studies to mixed cultures (neuroblastoma plus astrocytes) as a prelude to studies on explants, in which specific cell species can be selectively "deleted" and effects on K-dependent Cl-transport evaluated. In the antitumor drug studies, implantation of an experimental tumor in the monkeys is planned, followed by evaluation of drug levels achievable therein.

Honors and Awards:

Tower, D. B.:

1. Invitation as guest of the Academy of Sciences, Armenian SSR, to present a paper on the role of astroglia as modulators of neuronal function at an International Neurochemical Symposium held in Yerevan, Armenian SSR, October 16-22, 1972.

2. Guest of the Academy of Sciences, Uzbek SSR, at Tashkent, October 24-26, 1972.

3. Invited guest of the American Association of Neurosurgeons (Harvey Cushing Society) to participate in a seminar on cerebral edema at the 1973 Los Angeles meeting.

Publications:

1. Tower, D. B.: Fluids and electrolytes in the central nervous system: factors affecting their distribution with special reference to excitability and edema. pp. 43-57 in Recent Contributions to Neurophysiology edited by J-P Cordeau and P. Gloor, Amsterdam, Elsevier, 1972.

2. Tower, D. B.: Cerebral edema. pp. 537-554 in Basic Neurochemistry edited by R. W. Albers, G. J. Siegel, R. Katzman and B. W. Agranoff. Boston, Little, Brown & Co., 1972.

3. Tower, D. B., and Young, O. M.: The activities of butyrylcholinesterase and carbonic anhydrase, the rate of anaerobic glycolysis, and the question of a constant density of glial cells in cerebral cortices of various mammalian species from mouse to whale. J. Neurochem. 20: 269-278, 1973.

Serial No. NDS(1)-61 LNC/EC 813
1. Neurochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Enzymological Aspects of Neural Function.

Previous Serial Number: Same.

Principal Investigator: R. W. Albers, Ph.D.

Other Investigators: S. Goldman, Ph.D.; D. H. Jean, Ph.D.; and G. J. Koval.

Cooperating Units: Section on Trophic Nerve Function, LNNS, NINDS;
Laboratory of Biophysics, NINDS.

Man Years:

| | |
|---|---|
| Total: | 5.5 |
| Professional: | 4.0 |
| Other: | 1.5 |

Project Description:

Objectives: To assess the functional roles, molecular structures and mechanisms of enzyme systems which characterize neural tissue or which respond to neural influences. Current studies are directed toward (1) the $Na^+$-$K^+$-ATPase which is the basis for the electrical potential developed across neurons, and (2) myosin ATPase which appear to be under neural control at the genetic level.

Methods:

General Methods - The relation of certain enzymes or enzyme systems to neural functions is usually deduced from neurophysiological or metabolic studies on intact organisms or relatively intact tissues. Observations on the effects of e.g. behavioral or pharmacological interventions as reflected in levels of metabolites or other tissue constituents may permit deductions as to their possible enzymatic basis.

Biochemical investigations in this section usually begin as an investigation into the existence of a particular enzyme or enzyme system hypothesized from physiological data. This may involve development or modification of an analytical method based upon the chemical properties of the reactants or products of the hypothetical enzyme reaction. Optical or radioisotope techniques are commonly employed.

If the initial hypothesis is confirmed, further explorations of the properties of the enzyme are designed to further test the correlation of properties of the enzyme with function and to elucidate the molecular bases of neural function. These studies ordinarily require some purification of the enzyme by conventional techniques of solubilization, salt fractionation,and chromatography.

Characterization of the purified enzyme by kinetic, physical and degradative analyses can lead to the formulation of various subsidiary hypotheses concerning e.g., physiological regulation, mechanisms of drug action or possible relationships to pathological processes.

Specific Methods - The Section on Enzymes has directed most of its attention for several years to the ($Na^+ + K^+$)-dependent ATPase which is the biochemical expression of the active $Na^+$ transport process.

The specific methods required for these studies have included development of several assay systems for the ATPase and related reactions, based on optical and radioisotopic principles. Since this enzyme system is an integral component of plasma membranes, we have extensively investigated and developed methods of solubilizing membrane proteins with detergents and organic solvents. The conventional protein purification methods are not applicable to these proteins. New purification procedures have been developed to be compatible with the use of detergents and organic solvents. Numerous other analytical procedures are utilized as required, e.g. for the analysis of phospholipids associated with the $Na^+$-$K^+$-ATPase.

Stopped-flow kinetic techniques are under development for application to projected studies of the mechanism of active $Na^+$ transport. These include direct digital data acquisition of spectral changes which occur on the millisecond time scale and computer processing of this data.

Digital data acquisition and processing methods are also under development for application to the quantitative evaluation of fluorescence probe studies and the quantitation of protein peaks on gel electrophoretic patterns.

The tryptic fragmentation method of distinguishing closely related proteins has been previously applied to studies of myosins; it is now being examined as a possible tool in studying the structure of the $Na^+$ ATPase.

Major Findings:

(a) Solubilization of $Na^+$-ATPase: The electrophorus ATPase has been obtained in a soluble form during the past year. The soluble enzyme is much less stable than the membrane-bound enzyme. This creates difficulties in further purification. However a study of the stability properties of the soluble enzyme has resulted in development of a stable form of the soluble enzyme and conditions have been worked out which permit the enzyme to be subjected to chromatographic and electrophoretic purification without loss of significant activity.

(b) Gel electrophoretic studies of the soluble enzyme have demonstrated that it consists of two different polypeptide chains of approximately 90,000 and 60,000 daltons. We had previously shown that the larger subunit is phosphorylated by ATP. The molecular weight of the intact enzyme is about 300,000 daltons. A structure consisting of two each of the 60,000 and 90,000 subunits would be consistent with these observations.

Work is in progress on the preparative scale separation of the two different subunits.

(c) Demonstration of sodium-specific ionophoric peptides associated with the $Na^+$-ATPase: Our previous studies of the sodium transport system have led us to conclude that the $Na^+$-ATPase must contain $Na^+$ "carrier" sites as intrinsic parts of the enzyme protein. Detection of such sites requires a sensitive system which could be applied to relatively heterogeneous materials and which could detect a low concentration of bound $Na^+$ in the presence of large amounts of free $Na^+$. The black lipid bilayer membrane (BLM) seemed to meet these criteria. If the $Na^+$ "carrier" can product $Na^+$-specific conductance in the BLM high sensitivity to the carrier in the presence of $Na^+$-containing electrolyte solution would be expected.

A collaborative project was initiated with Dr. Adil Shamoo (Mt. Sinai Medical School, Dept. of Physiology and Biophysics), a guest worker in the Laboratory of Biophysics, NINDS.

This project has demonstrated that tryptic digests of the $Na^+$-ATPase do contain material which can confer $Na^+$-dependent conductance increased in the BLM system. Characterization of this material is in progress.

(d) Fluorescent probe studies of the $Na^+$-ATPase: Dr. Goldman has initiated a study of the interaction of amphipathic fluorescent molecules with the ATPase. These studies provide information about the nature of the exposed surfaces of the enzyme protein. One of the most widely used fluorescent probe molecules is anilino-naphthalene sulfonate which is essentially non-fluorescent in an aqueous environment but fluoresces intensely in organic solvents or when adsorbed to non-polar surfaces. The extent of binding and the fluorescence intensity provide information about the extent and degree of hydrophobic surfaces of proteins. In the case of a protein such as the ATPase, which evidently undergoes some conformational changes as a result of ligand binding, ligand binding may induce changes in the fluorescence emission of the bound dye.

Results to date indicate that the dye is bound to regions of relatively high polarity in the case of ANS interactions with the ATPase. Both low and high affinity binding has been detected. Binding is predominantly to protein surfaces since removal of phospholipids by phospholipases does not markedly change the binding.

Conformational changes in response to ligand binding appear not to be reflected in the ANS-ATPase interactions.

(e) Effect of myosin light chains on tryptic fragmentation of myosin heavy chains. Our previous studies have demonstrated that the tryptic fragmentation technique applied to myosins from fast and slow skeletal muscles yields different peptide patterns. The myosins used in this work contained both light and heavy chains. Since the light chains differ in the two myosins, the possibility existed that the light chain interactions conferred differences in tryptic susceptibility to heavy chain myosin which might be independent of the primary structure of myosin heavy chains.

This possibility has now been tested by preparing myosin heavy chains free of the light chains. When the heavy chains from slow and fast myosins are subjected to tryptic fragmentation, different patterns are still observed. This substantiates our original hypothesis that the two myosin heavy chains are products of different genes.

Significance: The enzyme systems currently under study are both of major functional significance with respect to the nervous system. The $Na^+$-ATPase is the biochemical expression of active $Na^+$ transport which is the primary process for maintaining the electrical activity of the brain. Myosin is the major contractile protein of muscle. As such, it reflects the neural control of muscle function as well as the physiological responses of muscle to use, disuse and disease.

Proposed Course: The availability of a stable, soluble $Na^+$-ATPase will facilitate structural and kinetic studies of the enzyme. Studies will be directed toward associating known functions of the enzyme with the two polypeptide chains. Separation of the two polypeptides will permit studies on possible reconstitution of the enzymatic activity.

Immunological studies should also be possible, using antibodies to the individual peptide chains. If cross-species reactivity can be obtained, the electrophorus preparation will afford a convenient source for the specific antigens which can be used for studies in more complex tissues such as brain.

Fragmentation studies of the purified peptides may be useful as a stage in working out the primary amino acid sequence of the enzyme.

The soluble enzyme will be particularly advantageous in the rapid reaction kinetic studies which are in progress. The low optical density of the preparation permits studies which were not possible with the membrane bound enzyme.

Continued collaboration in the characterization of the ionophoric material is projected. Dr. Shamoo will join the faculty at the University of Rochester in July and will continue to carry out BLM studies on material supplied by this laboratory.

Publications:

1. Albers, R. W., and Koval, G. J.: Sodium-potassium-activated adenosine triphosphatase of electrophorus electric organ. VIII. Monovalent cation sites regulating phosphatase activity. J. Biol. Chem. 248: 777-784, 1973.

2. Goldman, S. S., and Albers, R. W.: Sodium-potassium-activated adenosine triphosphatase. IX. The role of phospholipids. J. Biol. Chem. 248: 867-874, 1973.

3. Jean, D.-H., Guth, L., and Albers, R. W.: Neural regulation of the structure of myosin. Exp. Neurol., in press.

4. Shamoo, A. E., and Albers, R. W.: $Na^+$-selective ionophoric material derived from kidney and electric organ membranes. Proc. Nat. Acad. Sci. (U.S.), in press, 1973.

Serial No. NDS(1)-67 LNC/PM 1480
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Metabolism of Neurohumoral Transmitter Substances in Marine Animals

Previous Serial Number: Same.

Principal Investigator: E. G. Trams, Ph.D.

Other Investigators: A. A. Benson, Ph.D.; V. Glisin, Ph.D.; S. Patton, Ph.D.

Cooperating Units: Scripps Institute of Oceanography, La Jolla, Calif.; Centre for Multidisciplinary Studies, Belgrade, Yugoslavia; Div. of Food Science, Pennsylvania State University; Mote Marine Laboratory, Sarasota, Fla.

Man Years:

Total: 0.2
Professional: 0.2
Other: 0

Project Description:

Objectives: To exploit the great variety and abundance and the biochemical specialization of oceanic life for the study of neurobiology. In particular we have selected to study some aspects of neurochemistry in marine organisms where certain tissues, species or phenomena constitute an evolutionary amplification of normal human physiology.

Methods: Our studies are mostly concerned with the metabolism of transmitter substances and the methodology in use is adjusted to specific requirements according to current laboratory techniques. In the field of neurotransmitter metabolism use is made of radioactive precursors; labeled biogenic amines are introduced for studies of their metabolism *in vivo* or *in vitro*. Methods which are common to all enzymological approaches are used where appropriate, especially for studies in drug metabolism, toxicology, enzyme deletions, etc.

Experimental animals or organisms used: various kinds of Selachians, Torpedinidae, several species of Teleosts, in particular *Electrophorus electricus* and Pacific salmon (Oncorrhyncus spp.); small crustaceans, Ecchinoderms, *Aplysia*. Emphasis has been placed on the study of bioelectric organs and nervous tissues or tissues which are of interest to our principal program on structure and function of excitable membranes.

Major Findings: We have made use of an excellent marine model for a study on the biosynthesis (and control thereof) of plasma membranes. During the first few hours after fertilization sea urchin embryos divide nearly synchronously. As the number of cells increases from 1 to $2^{10}$, plasma membrane volume increases about tenfold. The increase in plasma membrane biomass presumably should be accompanied by a proportional increase in membrane marker molecules.

The following presumptive plasma membrane "marker" enzymes were assayed in whole homogenates of sea urchin cultures as well as in plasma membrane enriched fractions: $Na^+$-, $K^+$-ATPase, $Mg^{2+}$ ATPase, 5'-nucleotidase, neutral p-nitrophenylphosphatase and adenylcyclase. Unesterified cholesterol was determined in whole cultures only. Adenylcyclase activity in whole homogenates remained constant (except for a brief post-fertilization increase) from the unfertilized egg to the swimming blastula stage. In membrane enriched fractions the activity of this enzyme increased. Reliable levels for $Na^+$-, $K^+$-ATPase activity were not obtained, 5'-nucleotidase activity could not be detected and the usefulness of $Mg^{2+}$-ATPase, p-nitrophenylphosphatase and unesterified cholesterol as plasma membrane markers in sea urchins was questioned.

It was concluded that the increase in plasma membrane biomass of one order of magnitude did not necessarily require an equivalent increase (or de novo synthesis) of its constituent molecules. The latter most probably exist pre-formed in the unfertilized egg and hence are of maternal origin during the early stages of development.

These studies were conducted in cooperation with the Department of Molecular Biology (Prof. Glisin, Head) of the Centre for Multidisciplinary Studies, Belgrade and with the aid of the staff of the Mote Marine Laboratory (Dr. P. Gilbert, Director).

During a two week research expedition aboard the R/V "Dolphin" (Scripps Institution of Oceanography and Foundation for Ocean Research, La Jolla, California) we conducted further studies on the aging process in Pacific salmon (Oncorhyncus sp.). Our primary goal was to obtain further data on the functional degeneration of the CNS of the salmon during spawning migration. Specifically we had planned to compare the activity of the $Na^+$, $K^+$ ATPase of the brain in healthy salt water fish with that of fish during the various stages of migration. Due to technical difficulties these experiments were completed only in part. At the same time we collaborated with Profs. Benson and Patton in a study on the energy metabolism of the salmon heart muscle during migration. High levels of triglycerides in heart muscle of migrating salt water salmon were found which contrasted sharply with the almost complete absence of this metabolic fuel in the spawned out animals. This brief study was mostly descriptive in character but it is planned to make a further collaborative effort by investigating the functional controls (hormonal, neuronal, etc.) which serve command functions over cardiac energy metabolism.

Significance: These investigations again emphasize the usefulness of marine organisms as models for biochemical or physiological phenomena where research with common laboratory animals either would be unfeasible or very expensive. Our study on plasma membrane biosynthesis in developing sea urchin embryos underscores two important points. 1. In rapidly dividing embryonic cells the molecular constituents of the plasma membranes appear to exist preformed in the cytoplasm. 2. They are incorporated (or transformed) into plasma membrane by a process of "structuralization", a process not unlike crystallization where molecules with a natural "fit" assemble into structures which are morphologically recognizable.

Proposed Course: Our attempts to isolate a fairly pure plasma membrane fraction from developing sea urchins have not been very successful and we have designed new experiments to overcome some technical problems. In particular we want to label the plasma membrane surfaces with isotopic concanavalin A. This would not only serve as a marker for plasma membrane fractions during preparative isolation but will also give some measure of the per cent recovery obtained. We estimate that in order to obtain a membrane fraction of about 50% purity, in the unfertilized egg we would have to attain a purification factor of about 10 . If such purification can be obtained we should be able to address ourselves experimentally to the mechanisms of "structuralization". In particular we shall follow the pathways by which preformed adenylcyclase is incorporated into the expanding plasma membrane mass.

We also hope to have an opportunity to continue our collaborative studies on the aging process in the Pacific salmon. Specifically we are planning to conduct some fatty acid turnover studies in salmon heart muscle.

As for some advance planning we are presently formulating a program for the R/V "Alpha Helix" for 1974 on the 'Pacific Salmon as a Model for the Aging Process' (in collaboration with Profs. A.A. Benson and S. Patton).

Honors and Awards: E. G. Trams, Visiting Scientist, Laboratory of Molecular Biology, Centre for Multidisciplinary Studies, Belgrade and Kotor, Yugoslavia, May-June 1972.

Publications:

1. Patton, S., and Trams, E.G.: Salmon heart triglycerides during spawning migration. *Comp. Biochem. Physiol.* (in press).

Serial No. NDS(1)-67 LNC/PM 1481
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on the Composition and Metabolism of Cellular Membranes.

Previous Serial Number: Same.

Principal Investigator: E. G. Trams, Ph.D.

Other Investigators: A. Solyom, M.D., Ph.D.; Carl J. Lauter.

Cooperating Units: None.

Man Years:

| | |
|---|---|
| Total: | 2.4 |
| Professional: | 1.9 |
| Other: | 0.5 |

Project Description:

Objectives: To elucidate the inter-relationship between the molecular composition of the plasma membrane and the functions that such building blocks serve in transport, bioelectrogenesis and inter-cellular communication.

Methods: Our basic methodology, as in previous years, consisted of the measurement of function and composition of molecular elements which are thought to be characteristic of plasma membranes. In our recent studies emphasis has been placed on assays for the so-called "plasma membrane marker enzymes". In particular, we were concerned with studies on phosphoesterhydrolases, i.e. ATPases, 5' nucleotidase, adenylcyclase, nucleotide pyrophosphatase, p-nitrophenylphosphatase, phosphodiesterase and glucose-6-phosphatase. For the enzyme 2', 3' cyclic AMP-3'-phosphoesterhydrolase we developed a new method which is based in the fluorometric determination of a fluorescent substrate synthesized by us: 1, $N^6$-ethenoadenosine-2',3'-cyclic monophosphate. The new method is considerably more economical than those presently in use and we estimate that its sensitivity exceeds other methods by about two orders of magnitude.

A significant departure from previous methodology was our extended use of tissue culture preparations. In earlier studies we had pursued the problem of plasma membrane marker enzymes mostly on plasma membranes isolated from rat or guinea pig livers. During the last year, however, our problem required not only the use of viable, intact cells but also of a variety of cell lines for comparative purposes. In the course of our recent studies we have employed embryonic mouse hepatocytes, human hepatocyte (Chang), human carcinoma of the cervix (HeLa), human carcinoma of the larynx (KB Eagle), human skin fibroblasts, mouse neuroblastoma (N-18) and embryonic hamster astrocytes.

In many instances our analytical methods had to be adapted for use with tissue cultures, either because of the smaller amounts of material to be assayed or because experiments had to be conducted in complex media or on actively metabolizing intact cells. By and large our methodological problems have been solved and we do not anticipate major difficulties in that respect.

Major Findings: The main theme of the Section on Physiology and Metabolism is the molecular basis of excitability. We have been concerned, therefore, with the biochemistry of neurotransmitters, receptors and plasma membranes. Although our current projects focus on the problems of plasma membrane structure and function we suggest that our future research may include a novel aspect; namely, that of inter-cellular communication. Our working hypothesis is, that certain plasma membrane enzymes play a role analogous to acetylcholinesterase and catechol-O-methyltransferase and that they participate in information transfer between neighboring cells.

As a result of our recent investigations we have come to the conclusion that the so-called "plasma membrane marker enzymes" are by no means characteristic for plasma membranes of eucaryotic cells in general. The physiological role of most of these enzymes in the plasma membrane is not understood but from our data and from a review of the literature it became clear that in most cells certain powerful phosphoesterhydrolases were the dominant, plasma membrane associated, macromolecules. Only two of the enzymes in this class serve a function which is consistent with our present concept of membrane biology: the $Na^+$, $K^+$ ATPase dealing with ion transport and adenylcyclase synthesizing the second messenger, 3', 5' cAMP. No functions have been assigned to the other phosphoesterhydrolases which have been identified in most cell membranes: i.e., 5' nucleotidase, phosphodiesterase, nucleotidepyrophosphatase and other ATPases.

In order to make functional assignments for these enzymes we focused our initial investigations on the problem of "sidedness". Namely, we have asked: which of these enzymes is associated with the external aspect of the plasma membrane and which with its internal aspect?

Our experimental approach was to measure enzymatic activity on intact cells--presumably an index of Ecto-enzyme activity and to compare this with data obtained on disrupted cells where presumably the inner (as well as the outer) face of the plasma membrane interacted with the substrates.

The most significant major finding which we wish to report here is that a definite pattern of "sidedness" has been shown to exist in the cell types so far investigated. We have found that the classical membrane marker enzyme, 5' nucleotidase is mostly, if not entirely, associated with the exterior aspect of the cell membrane. Permeation of the conventional substrate (5' AMP) through the membrane of intact cells did not occur. Kinetic studies on this enzyme with a variety of substrates indicated that it has a low substrate specificity and that phosphomonoesters other than those of nucleosides show mixed inhibition. Comparative studies on intact cells further showed that the specific activity of 5' nucleotidase varied more than 20-fold (i.e., KB cells 2.19, HeLa 0.084 μmoles/hr/$10^6$ cells).

Another active enzyme on the cell surface is a non-specific ATPase. This enzyme is not related to the $Na^+$, $K^+$ ATPase of the sodium pump. Ouabain, a classical inhibitor for the latter, had no effect on its activity. From studies with a variety of nucleosidedi- and triphosphates we concluded that the specificity of the enzyme for ATP was not very high. On the other hand, the specific activity of this enzyme is rather high in most cell lines. As a result it has been rather difficult to study the effects of nucleosidetriphosphates on isolated cells. We have conducted a few such experiments because of some preliminary observations that extrusion of nucleosidetriphosphates and reuptake of their metabolites occurred.

One enzyme which was clearly located on the interior membrane aspect is adenylcyclase. This finding, of course, agrees well with our teleological interpretation of the function of adenylcyclase. Another enzyme, nucleotidepyrophosphatase, appears to have a mixed but preferential "inside" localization as judged from a series of experiments. Our data on p-nitrophenyl-phosphatase, phosphodiesterase I and leucyl-β-naphthylamidase are still too preliminary for generalized conclusions. If succinic dehydrogenase (a mitochondrial enzyme) was assayed as a control on intact cell preparations no activity was observed, as expected.

Although the pattern of enzymic activity was qualitatively similar in all cell lines, fairly substantial quantitative differences were observed and at the present time we have not been able to draw any conclusions about the functional significance of enzyme distribution and activity in plasma membranes.

Significance: We are of the opinion that these findings represent a significant advance in the understanding of plasma membrane biology. Our observations strongly support the notion of a molecular asymmetry of plasma membrane structure. While this concept has long been accepted by physiologists, supporting biochemical data have been accepted by physiologists, supporting biochemical data have been scarce.

In addition, we believe that our observations can serve as departure points for further investigations on the function of these enzymes. Our working hypotheses for projected experiments, to a large extent, have been based on our recent findings.

Proposed Course: We plan to extend our studies on plasma membrane marker enzyme "sidedness" to include several other cell types. Additional experiments will be designed to inquire if culture conditions, growth patterns, absorptive states, contact inhibition, malignancy, etc., influence or change the observed pattern of enzyme distribution. Attempts will be made to identify the natural substrates for these enzymes. Our long-range goal continues to be the description of the molecular biology of excitable membranes.

Publications:

1. Solyom, A., Lauter, C. J., and Trams, E. G.: Plasma membranes from isolated liver cells. *Biochim. Biophys. Acta* 274: 631-637, 1972.
2. Solyom, A., and Lauter, C. J.: A study of sex differences in enzyme activities of rat liver plasma membranes. *Biochim. Biophys. Acta*, in press.
3. Solyom, A., and Trams, E. G.: Enzyme markers in characterization of isolated plasma membranes. (Review) *Enzyme*, in press.

# ANNUAL REPORT
July 1, 1972 through June 30, 1973
Laboratory of Perinatal Physiology
National Institute of Neurological Diseases and Stroke

Ronald E. Myers, M.D., Ph.D., Chief

The Laboratory of Perinatal Physiology continues to focus upon perinatal brain damage and its pathogenesis using a rhesus monkey experimental model. In addition, the Laboratory is concerned with pregnancy abnormalities which may adversely affect brain development either through producing destructive lesions or altering its formation. Secondly, the Laboratory carries out studies within experimental neuropathology, focusing on severe energy deprivation states (of nervous system tissue). These states include asphyxia, hypoxia, hypoglycemia, hypotension, cyanide, carbon monoxide, and still other circumstances. Particular attention is paid to the patterns of brain injury produced and the mechanisms through which these patterns occur. Neuropsychology is the third area of Laboratory concern. Efforts here explore the neurology of emotion and social behavior.

The pathophysiology and pathogenesis of perinatal brain damage has been clarified to a considerable extent by work in the Laboratory. The levels of oxygen deprivation required to bring about alterations in cardiovascular and nervous system function in term monkey fetuses have been defined with precision. Major progress has also been made in defining the relationships between a variety of patterns of brain injury which are described in the human and their mechanisms of occurrence. It now seems clear that the earliest change which occurs with severe oxygen deprivation is a dramatic shift of electrolyte and fluid distribution between the various spaces of the brain. Water is also taken up by the brain as a whole. These alterations lead to brain swelling, impairments in local tissue capillary blood circulation, and tissue necrosis. With survival these zones of necrosis are converted into zones of ulegyria, white matter sclerosis, status marmoratus of basal ganglia, porencephaly, and cystic brain degeneration.

A major program has been concluded which has provided insight into the relation between asphyxia (oxygen deprivation) and the vital signs changes in the fetus as may be monitored through fetal electrode placements. It is now clear that episodes of fetal bradycardia which follow uterine contractions are indicative of late degrees of fetal asphyxia. It is also clear that such episodes of bradycardia depend in their conformation both on the severity of asphyxia and on the durations of the uterine contractions. These studies have led to an understanding of the significance of a variety of patterns of bradycardia and their relation to both fetal asphyxia and potential brain injury.

The effects on the fetus of the use of prostaglandins and of adrenergic blocking agents have been studied. This knowledge is critically important since prostaglandins have been proposed for use in the induction of labor, and adrenergic blocking agents are increasingly prescribed in pregnant women for the control of hypertension. These studies have shown that use of these drugs, even in dosages normally prescribed, may adversely affect the fetus, usually producing fetal asphyxia. Such effects are particularly prominent when, for one reason or another, the fetal state is already in some jeopardy. The mechanisms through which these powerful drugs act to produce these effects on the fetus include alterations in uterine perfusion produced by alterations in uterine blood vessel caliber and also alterations in cardiac output of the mother.

Fetal death following intra-amniotic hypertonic saline solution infusion (increasingly used to produce midgestational abortions) occurs by producing a dramatic congealing of the fetal blood in the smaller caliber umbilical blood vessels on the placental surface. This blood congealing leads to immediate fetal asphyxia and fetal death. Significant alterations also were found in the uterine wall circulation with evidence for muscle wall necrosis. Prostaglandin instillation, on the other hand, for the induction of midterm abortion, acts by strongly stimulating the uterus and producing fetal asphyxia by secondarily impairing intervillous space perfusion.

Carbon monoxide, when inhaled by the pregnant monkey, leads to severe fetal hypoxia and fetal death because of its preemption of hemoglobin binding sites for oxygen. This progressive occlusion of oxygen binding leads to an impaired oxygen-carrying capacity of the maternal blood and a hypoxia of the fetus. Only small quantities of carbon monoxide are transmitted to the fetus because of the profoundly low partial pressures of CO produced in the maternal blood.

Carbon monoxide inhalation by juvenile rhesus monkeys produces symmetrical zones of white matter necrosis. This pathology pattern resembles that seen in the human under these same circumstances. However, the identical pattern of white matter injury occurs, both in human and rhesus monkey, under a variety of other circumstances and represents one of the important patterns of nervous system reaction to injury. Studies are under way attempting to elucidate the pathogenesis of this pattern of injury.

Careful correlative work with a variety of models of brain damage which have been worked with in the Laboratory have indicated generally a poor correlation between the absolute values of the hypoxia in the juvenile animal and the occurrence and severity of brain pathology. Studies currently under way with carefully regulated exposures of juvenile monkeys to hypoxia have shown a similar variable relationship between the absolute oxygen values in the juvenile blood and the occurrence and severity of the patterns of altered cardiovascular performance. That is, the first appearance of lowering of blood pressure among different animals varies considerably in its relationship to the corresponding partial pressures of oxygen in the blood. Thus, different animals respond to the same oxygen deprivation in quite dissimilar ways not only with respect to brain pathology generated but also in changes in cardiovascular performance. This critical point is currently under further investigation.

The effects of severe hypotension on the nervous system is under careful study. Hypotension is produced using ganglionic blocking agents (Arfonad), bringing the mean blood pressure down to 30 ± 2 mm Hg. Juvenile monkeys were subjected to hypotension for 15- and 30-minute periods. After 15 minutes, a small proportion, and, after 30 minutes, a large proportion of animals die later of inability to maintain blood pressure despite use of pressor agents. Nonetheless, animals which survive generally show no brain pathology. During and immediately following these episodes, the EEG may show decreases in amplitude and frequency but rarely does flattening appear. However, during the recovery period, brief periods of EEG flattening may be precipitated by small barbiturate injections. Impedance increases (of up to 100%) develop within minutes of the onset of hypotension suggesting rapid early fluid shifts from the extra- to the intra-cellular spaces. Paradoxically, these impedance changes are not necessarily associated with flattening of the EEG. The cisternal fluid pressures remain unaltered during hypotension. However, on restoring blood pressure with the use of pressor agents, the cisternal pressures rise by 50-400%. The cisternal pressure rises bear little relation to the ultimate survival of the animal. Fifteen minutes after the onset of hypotension, the glucose levels in serum and cisternal fluid begin to rise and this increase continues even after restoration of blood pressure. In animals which survive, the glucose levels remain elevated at 2-3 times normal for many hours, whereas those animals which later die show massive increases (10 X) in both blood and cisternal fluid glucose levels. Thus, blood pressure levels of 30 mm Hg still provide for cerebral perfusion to the extent that significant numbers of animals survive without damage, but such pressure levels do cause significant numbers of deaths due to alterations in subsequent performance of the heart.

Juvenile monkeys have been subjected to episodes of hypoxia where the $pO_2$s have ranged from 10-30 mm Hg. The rhesus monkey can sustain $pO_2$ levels as low as 20 mm Hg for varying lengths of time without significant or lasting sequelae. However, when the $pO_2$s are reduced to 15 mm Hg, the animals die when the exposures exceed 15 minutes. Exposure to a $pO_2$ of 10 mm Hg leads to rapid death in circulatory failure.

To more fully characterize the cardiovascular performance of rhesus monkeys subjected to various energy-depriving states, a computerized system has been devised which enables a constant monitoring of such parameters as cardiac contractility, cardiac output, and peripheral vascular resistance. Together with the Laboratory of Applied Studies of the Division of Computer Research and Technology, a system was devised where analog signals of blood pressure, left-ventricular pressure, EKG, heart rate, and dP/dT are recorded directly on magnetic tape. This tape is then digitized and used as input to the IBM-360 computer system. From this data and the appropriate programming, the computer can derive all these indexes of circulatory function at any time during the experiment. The accuracy and reproducibility of such measurements are extreme, thereby allowing a more precise analysis of pressure waveforms compared with the usual analog methods. Studies have been completed involving the effects of the frequency response of the recording system on indexes of contractility, the effect of phase shifts introduced by analog differentiators, the correlation of the components of fourier analysis of the pressure waveforms with various circulatory states. A detailed account of the cardiovascular response to severe hypoxic and anoxic insults has been studied. In this manner, the circulatory component to the overall physiological and neuropathological results can be ascertained. Measurements are being studied from the recovery phase after such insults to see if survivability can be predicted on the basis of a detailed knowledge of the state of the circulation, substantially before the final outcome is certain.

In biochemistry, studies have continued on the brain glycogen response to metabolic and other challenges. Twelve and one-half-minute episodes of total asphyxia lead to dramatic changes in glycogen content of monkey newborn brain. During asphyxia, the glycogen levels drop to profoundly low values and following resuscitation again increase to reach two times normal values by 12 hours. Thereafter, the brain glycogen gradually returns to normal. All durations of total asphyxia up to 9 minutes fail to produce these postasphyxial increases in glycogen, while all episodes beyond 9 minutes lead to the same maximal postasphyxial glycogen elevations. Thus, this postasphyxial brain glycogen response is an all-or-none process and has a threshold asphyxial duration of 9 minutes.

Episodes of severe hypoglycemia (glucose levels below 20 mg percent for 6 hours) also lead to dramatic glycogen increases in the juvenile monkey brain following restoration of blood glucose levels. The glycogen may double with maximal values appearing only after 24 hours. Thereafter, again, the brain glycogen content restores toward normal. Similar but more rapidly evolving changes occur in the juvenile following recovery from circulatory arrest and other severe energy deprivative states.

Starvation leads to significant alterations in glycogen content of brain and other organs. The liver glycogen decreases to 10% normal during the first 24 hours. By the end of the second day, only 5% of normal liver glycogen remains. Other organs, such as heart and muscle, show relatively little change during the first three days of starvation but, thereafter, deplete up to 50%. The brain, alone of organs, shows glycogen increases up to 50% with starvation. The highest values occur on the second day and, with restoration of feeding, the brain glycogen levels gradually restore to normal. The blood glucose level remains normal throughout.

The behavior of electrolytes is under study in relation to episodes of total asphyxia. Potassium increases both in the serum and the cortical subarachnoid fluid (4.5 → 8 mEq/L in serum and 3 → 18 mEq/L in CSF). Following resuscitation both serum and cortical subarachnoid fluid potassium levels return to normal by 15 minutes. While sodium in the serum shows a variable behavior, that in the cortical subarachnoid fluid remains unaltered. The glucose shows increases of 30-50% in serum and 20-30% in CSF during asphyxia. After resuscitation the glucose behavior varies according to whether epinephrine is administered. With epinephrine, the glucose content of both CSF and serum show major increases and remain elevated for hours, whereas, in resuscitation without epinephrine, only moderate glucose increases appear, peaking at 30-45 minutes. However, when epinephrine is injected into unasphyxiated animals, only slight alterations appear in serum glucose levels. Thus, asphyxia dramatically alters the blood glucose response to epinephrine stimulation.

Work has been completed on the role of the neocortical commissures in delayed response and delayed alternation performances. Since these functions largely depend upon the prefrontal cortex regions, it was presumed that the transfer of learning of these activities between the two sides of the body would be carried out by the anterior corpus callosum. However, animals with optic chiasma and corpus callosum transected still showed evidence for significant training transfer. However, initial depressions in performance of the commissure-operates on transfer testing suggested that the commissures still may transmit some information between the hemispheres within this mode of learning. The hypothesis is expressed that training transfer of these tasks, despite absence of the commissures, may relate to their strong dependency on memories of prior patterns of motor response (which has earlier been demonstrated in this Laboratory to survive commissure transection). Studies with a Go, No-go discrimination task, which again depends upon prefrontal cortex but which also is supported by specific visual cues, was totally interrupted by commissure transection. It was believed the critical effect preventing transfer in this instance was the non-transferability of specific visual information.

Separate studies were completed on the functions of the anterior temporal cortex. Other studies (in our Laboratory) with social behavior have pointed out striking similarities between deficits produced by lesions of prefrontal and of anterior temporal cortex. Thus, the anterior temporal cortex also may show other deficits which may be produced by lesions of prefrontal cortex. Rhesus monkeys were taught delayed response, delayed alternation, position discriminations, and a variety of extinction tests. Lesions of anterior temporal cortex failed to disturb performance on any of these tasks. However, visual discrimination learning, Go, No-go discriminations, and discrimination reversal functions were disturbed by the anterior temporal lesions. Thus, anterior temporal cortex lesions fail to show many of the deficits which occur after prefrontal lesions. The anterior temporal cortex, though it shares many functions with the prefrontal cortex, still has many unique functional aspects of its own.

Serial No. NDS(I)-66 LPP 1387
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Experimental placental abruption in the rhesus monkey and its relation to brain damage

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Prof. Dr. Maurice Panigel

Cooperating Units: Hopital St. Antoine, Paris, France

Man Years:

| | |
|---|---|
| Total: | .40 |
| Professional: | .08 |
| Other: | .32 |

Project Description: Project completed

Honors and Awards: None

Publications:

Myers, R. E.: The gross pathology of the rhesus monkey placenta. J. Reprod. Med. 9: 171-198, 1972.

Myers, R. E., and Panigel, M.: Experimental placental detachment in rhesus monkey: Changes in villous ultrastructure. J. Med. Primatol. In press.

Serial No. NDS(I)-66 LPP 1388
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Perinatal asphyxia in the monkey and its CNS consequences

Previous Serial Number: Same and incorporating Serial No. NDS(I) 72-LPP 1953

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Myron D. Ginsberg, M.D.
Karlis Adamsons, M.D.
Michael E. Selzer, M.D., Ph.D.
Stanley B. Holstein, M.D.

Cooperating Units: Mt. Sinai School of Medicine, New York

Man Years:

| | |
|---|---|
| Total: | .40 |
| Professional: | .08 |
| Other: | .32 |

Project Description:

Objectives: To determine the patterns of clinical and neurological change produced by asphyxia during pregnancy or at the time of birth. To attempt to correlate the patterns of neuropathological change produced with the functional changes occurring in the fetus during asphyxia.

Methods Employed: Monkey fetuses are delivered by cesarean section and subjected to asphyxia prior to first gasp by placing a rubber sac over the head and clamping the umbilical cord. The lengths of asphyxia are of predetermined length or are determined by the degree of functional deterioration in the fetus. In some instances, an effort was made to produce the maximum degree of damage compatible with survival. Alternatively, the fetuses are subjected to episodes of partial asphyxia which may be produced by a variety of techniques, all of which impair intervillous space perfusion. Studies are carried out on the cardiovascular, blood chemical (acid-base status), blood gas, lactate, etc., and respiratory changes of the fetus during and after asphyxia. Resuscitation is carried out using positive pressure oxygen ventilation.

Major Findings: With the varying lengths of asphyxia, varying degrees of brain damage were produced. The most common clinical alteration observed was a sensory loss in the extremities and over the head, alterations in sucking and swallowing, alterations in vocalization, and varying degrees of ataxia and motor clumsiness. In some instances, trophic sores occurred in the extremities or over the face and head. With more severe damage, the animals showed changes in postural tone and in the use of extremities. In the more severe instances, a total paralysis and a severe flexor hypertonus affected all extremities. Pathologically, destructive changes affected the thalamus. Damage to cortex or basal ganglia appeared only in animals showing evidence of prior asphyxia.

Episodes of partial asphyxia failed to lead to lower brain stem patterns of injury and, instead, affected hemispheral structures including the cortex, the underlying white matter, and the basal ganglia. These latter patterns of brain injury closely resemble the brain damage observed in the human with perinatal asphyxia.

Significance: The intent is to reproduce the patterns of neuropathological change seen in cases of human cerebral palsy and mental retardation. To a great extent, the study has been fruitful in this regard. More important still is the continuing effort to determine the critical parameters involved in the functional deterioration of the fetus during asphyxia and to relate these physiological changes to the patterns of neuropathological change produced. Finally, efforts are being made to determine techniques through which the functional deterioration of the fetus may be corrected during asphyxia so as to prevent or postpone the occurrence of brain injury during labor.

Proposed Course of Project: We hope to extend the number of physiological and blood chemical changes which are measured in relation to the asphyxia. Efforts will also be made to separately alter the various functions in the fetus to determine their relative importance in the pathogenesis of perinatal brain damage.

Honors and Awards: None

Publications:

Myers, R. E., and Bito, L. Z.: Ontogenesis of blood-brain-barrier function in primate: CSF cation regulation. In Comline, K. S., Cross, K. W., Dawes, G. S., and Nathanielsz, P. W. (eds.): Sir Joseph Barcroft Centenary Symposium on Foetal and Neonatal Physiology. Cambridge, England, 1973, pp. 40-48.

Myers, R. E.: Fetal asphyxia due to umbilical cord kinking: Combined hemisphere and brain stem injury. Perinatology. In press.

Myers, R. E., Valerio, M. G., Martin, D. P., and Nelson, K.: Perinatal brain damage: Porencephaly in a cynomolgous monkey. Biol. Neonate. In press.

Serial No. NDS(I)-67 LPP 1464
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemistry of the recovering rhesus monkey from various different states of energy deprivation

Previous Serial Number: Same

Principal Investigator: Americo Rivera, Jr., Ph.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

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|---|---|
| Total: | 2.40 |
| Professional: | 1.08 |
| Other: | 1.32 |

Project Description:

Objectives: To study the effects of energy deprivation on the glycogen levels of the brain and other tissues of the rhesus newborn and juvenile monkey. The project includes investigations on:

a) The glycogen changes in brain and other tissues of the juvenile monkey recovering from circulatory arrest.

b) The glycogen levels of brain and other tissues of the newborn rhesus monkey recovering from an episode of prolonged partial asphyxia.

c) The effects of barbiturate anesthesia on brain glycogen in the monkey.

d) The glycogen levels in the tissues of recovering acutely asphyxiated newborn monkeys.

e) The glycogen levels of the brain and other tissues of the juvenile recovering from a 6-hour episode of hypoglycemia (< 20 mg % of blood glucose).

These investigations have been expanded to include the determination of electrolytes in the spinal fluid from the subarachnoid space, cisterna magna and lumbar area.

Methods Employed: The deprivation of energy to the brain of the various experimental animals has been accomplished by 1) oxygen deprivation (asphyxia, acute or partial), 2) systemic ischemia, 3) prolonged deep anesthesia, and 4) hypoglycemia.

The brain, heart, lung, liver, spleen, kidney, and muscle (adductor) are rapidly excised from the anesthetized animals at various time intervals of the recovery period and frozen in a mixture of isopentane-isohexane cooled to the temperature of liquid nitrogen. The glycogen levels of these tissues were determined.

Major Findings: It was found that 12 hours following the asphyxia or ischemia episode the glycogen level in the brain peaks to approximately twice the control level. This maximum glycogen content of the brain 12 hours after the insult is being used to assay the threshold amount of asphyxia necessary to bring about this response. Using this criterion, it has been found that 9 minutes or more of asphyxia will produce cerebral glycogen accumulation after 12 hours. Less than 9 minutes of asphyxia gives no response. More than 9 minutes of asphyxia does not increase the response. This all-or-none response is triggered by the complete depletion of the cerebral glycogen during the asphyxia.

The hepatic glycogen is not affected by the various energy deprivation states. The levels of glycogen of the liver reflect the dietary status of the animals. The heart, lung, kidney, and muscle recovered rapidly and showed no residual effect as far as their glycogen content is concerned. The muscle and kidney glycogen of the juvenile animals are less tolerant to episodes of anoxia than the newborns.

The cerebral glycogen levels of the fetus are not as sensitive to prolonged partial asphyxia as polysome integrity and protein synthesis. Prolonged partial asphyxia does not result in a decrease in cerebral glycogen if the blood pH did not drop below 7.0.

It has been found that brain glycogen steadily increases to 170% above normal values over the first 48 hours under anesthesia, in spite of the severe restrictions on caloric intake. The increase in brain glycogen due to restricted caloric intake (starvation) is only 32%. This cerebral glycogen response to starvation is triggered by depleted hepatic glycogen levels. The glycogen buildup in the brain of anesthetized monkeys follows a different pattern than that seen for asphyxia and ischemia.

Unlike acute asphyxia, ischemia, and anesthesia in the monkey, the brain of hypoglycemic monkeys tends to have its glycogen levels increase steadily for 24 hours and level off at 150% above the control levels. At 60 hours the glycogen levels are still very high. The other tissues show no changes in glycogen concentration over the controls. The rate of synthesis of cerebral glycogen during the recovery period is one-half that found in the monkeys recovering from asphyxia and ischemia. This might be related to the complete breakdown of the polysomes of the brain during the hypoglycemia so that there is a marked decrease in the protein synthetic capacity of this tissue.

Significance: The glycogen response by the brain 12 hours after the onset of an asphyctic or ischemic episode is more sensitive than the morphological changes. The carbohydrate changes are reversible.

The muscle and kidney of the juvenile animals also show alteration in their storage patterns during the recovery period. These investigations have pointed out that different forms of energy deprivation result in remarkably similar changes in the carbohydrate metabolism of the brain. These changes appear reversible.

Proposed Course of Project: Investigation of the enzymes of the glycogen biosynthesis pathways will be studied, as well as the ATP and electrolyte levels of the spinal fluid, and to ascertain if the mechanisms of the changes can be elucidated.

Honors and Awards: None

Publications:

Rivera, A., Jr., Miller, J. R., and Martinez-de Jesus, J.: Glycogen of the brain and vital organs of monkeys recovering from systemic ischemia. Amer. J. Physiol. In press.

Serial No. NDS(I)-69 LPP 1699
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Functions of forebrain commissures in learning

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Kazuo Hara, Ph.D.
Shun-ichi Yamaguchi, Ph.D.

Cooperating Units: Department of Psychology, International Christian University, Mitaka, Tokyo, Japan

Man Years:

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| Total: | 1.08 |
| Professional: | .42 |
| Other: | .66 |

Project Description:

Objectives: To determine the functional role of the forebrain commissures in interhemispheric transfer of learning. Specific questions addresaed are: the degrees of transfer deficits in relation to the complexity of visual stimuli and the difficulty of discrimination problems (Exp. I); timing of the commissurotomy in relation to the progress of learning (Exp. II); and the degree of early sensory deprivation (Exp. III).

Methods Employed: Exp. I. Midsaggital sectioning of the optic chiasma, the forebrain commissures: learning of visual discrimination by one eye, while the other eye is occluded, transfer test after shifting the occluder, reversal learning and subsequent second transfer test with the opposite eye. Five different discriminanda are studied--brightness, color, pattern, form, and scene.

Exp. II. General procedure is the same as the Exp. I, except that discriminanda utilizing different stimuli are presented in random order. Midsaggital sectioning of the corpus callosum and the anterior commissure is postponed until the subjects complete initial learnings of the third problem.

Exp. III. During various postnatal periods, infant monkeys are subjected to midline sections of the optic chiasma, the anterior commissure and the corpus callosum. At the same time, the eyelids of one eye are sutured closed. These subjects are raised with monocular vision for twelve months, and the closed eye then reopened. The testing procedure as employed for the Exp. I will be followed.

Major Findings: Exp. I. Monkeys with total section of the forebrain commissures and the optic chiasma exhibited moderate degrees of interocular transfer of brightness, color, and scene, while no such transfer in pattern and form was observed. Those stimuli which manifest transfers are available in the natural habitat and thus have become components of social signal, which might be handled in a quantitatively different fashion than neutral signals by the visual mechanisms.

Exp. II. In contrast with the evidence obtained with respect to intermanual transfer of tactual learning, midsaggital section of the forebrain commissures after learning by one side was followed by some absence of transfer.

Exp. III. The analysis of the results has not been completed.

Significance: Though the lack of interocular transfer of visual learning after midline section of commissural fibers has been observed by many investigators, many ambiguities on the nature of those deficits still remain. The present study aims to clarify some of the issues which relate to such variables as the difficulty of the discrimination problems and the complexity of the stimuli as well as the degree of sophistication of subjects and the interproblem transfer or generalization of learning tasks. Also, the last experiment would provide critical tests for the time course of development of visual perception and visual learning and of commissural functions in relation to these processes. Findings from these studies have relevance for our understanding of the rehabilitation of human blindness (*amblyopia ex anopsia*) as well as for the psychological understanding of sensory deprivation and its effects.

Proposed Course of Project: All of the specific experiments described have been completed. To plan and continue further experiments.

Honors and Awards: None

Publications:

Myers, R. E.: The forebrain commissures and their functions. In *Cerebral Interhemispheric Relations*, International Colloquium held in Smolenice, June 1969. Bratislava, Czechoslovakia, Publishing House of the Slovak Academy of Sciences, 1972, pp. 15-20.

Yamaguchi, S., and Myers, R. E.: Age effects on forebrain commissure section and interocular transfer. *Exp. Brain Res.* 15: 225-233, 1972.

Yamaguchi, S., and Myers, R. E.: Prefrontal lobe functions and the forebrain commissures. *Exper. Brain Res.* In press.

Serial No. NDS(I)-69 LPP 1715
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effect of hypoxic hypoxia on the monkey brain

During FY 1973 this project was incorporated with Serial No. NDS(I)-73 LPP 2020.

Serial No. NDS(I)-70 LPP 1810
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Vital signs of the fetus as indicators of the state of fetal oxygenation

Previous Serial Number: Same

Principal Investigator: Karlis Adamsons, M.D., Ph.D.

Other Investigators: E. Mueller-Heubach, M.D.
Ronald E. Myers, M.D., Ph.D.

Cooperating Units: Mt. Sinai School of Medicine and College of Physicians and Surgeons of Columbia University, New York, New York

Man Years:

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|---|---|
| Total: | .40 |
| Professional: | .08 |
| Other: | .32 |

Project Description: Project completed

Honors and Awards: None

Publications:

Adamsons, K., and Myers, R. E.: Perinatal asphyxia: Causes, detection and neurological sequelae. Pediat. Clin. of North America. In press.

Myers, R. E., Mueller-Heubach, E., and Adamsons, K.: Predictability of the state of fetal oxygenation from quantitative analysis of components of late deceleration. Amer. J. Obstet. Gynec. In press.

Serial No. NDS(I)-70 LPP 1818
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Experiences with a large breeding colony of *Macaca mulatta*

Previous Serial Number: Same

Principal Investigators: Ronald F. DiGiacomo, V.M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | .40 |
| Professional: | .08 |
| Other: | .32 |

Project Description: Project terminated

Honors and Awards: None

Publications:

DiGiacomo, R. F., and Missakian, E. A.: Tetanus in a free-ranging colony of *Macaca mulatta*: A clinical and eipzootiologic study. *Lab. Anim. Sci.* 22: 378-383, 1972.

Morrison, J. A., and Menzel, E. W., Jr.: Adaptation of a free-ranging rhesus monkey group to division and transplantation. In Krumholz, L. A. (ed.): *Wildlife Monographs No. 31*. Washington, D. C., The Wildlife Society, 1972, pp. 1-78.

Serial No. NDS(I)-70 LPP 1824
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neurology of vocalization in rhesus monkeys

During FY 1973 this project was incorporated with Serial No. NDS(I)-73 LPP 2022.

Serial No. NDS(I)-71 LPP 1899
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical and neuropathological features of experimental carbon monoxide intoxication in the primate

Previous Serial Number: Same

Principal Investigator: Myron D. Ginsberg, M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | .40 |
| Professional: | .08 |
| Other: | .32 |

Project Description: Project completed

Honors and Awards: None

Publications:

Ginsberg, M. D., and Myers, Ronald E.: Clinical and neuropathological aspects of carbon monoxide intoxication in the primate: Production of a leukoencephalopathy. Trans. Amer. Neurol. Ass. 97: 207-211, 1972.

Serial No. NDS(I)-71 LPP 1901
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neuropathological effects of sodium cyanide in rhesus monkeys

During FY 1973 this project was incorporated with Serial No. NDS(I)-73 LPP 2020.

Serial No. NDS(I)-71 LPP 1902
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neuropathological, physiological and metabolic effects of systemic hypotension on adult monkey brain

During FY 1973 this project was incorporated with Serial No. NDS(I)-73 LPP 2020.

Serial No. NDS(I)-72 LPP 1953
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The physiologic and neuropathological consequences of maternal carbon monoxide exposure on the term fetus

During FY 1973 this project was incorporated with Serial No. NDS(I)-66 LPP 1388.

Serial No. NDS(I)-72 LPP 1954
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neural structures involved in vocalizations of the cottontop marmoset

Previous Serial Number: Same

Principal Investigator: Elizabeth F. Ratliff, M.A.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | .98 |
| Professional: | .08 |
| Other: | .90 |

Project Description: Project terminated

Honors and Awards: None

Publications: None

Serial No. NDS(I)-72 LPP 1955
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Importance of corpus callosum in modification of affective behavior in rhesus monkeys with sectioned optic chiasma in combination with specific cortical lesions

Previous Serial Number: Same

Principal Investigator: Elizabeth Franzen Ratliff, M.A.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | .98 |
| Professional: | .08 |
| Other: | .90 |

Project Description: Project completed

Honors and Awards: None

Publications:

Franzen, E., and Myers, R. E.: Neural control of social behavior: Prefrontal and anterior temporal cortex. *Neuropsychologia*. In press.

Franzen, E. A., and Myers, R. E.: Age effects on social behavior deficits following prefrontal lesions in monkeys. *Brain Res*. In press.

Myers, R. E.: Role of prefrontal and anterior temporal cortex in social behavior and affect in monkeys. *Acta Neurobiol. Exp*. 32: 567-579, 1972.

Myers, R. E., Swett, C., and Miller, M.: Loss of social group affinity following prefrontal lesions in free-ranging macaques. *Brain Res*. In press.

Serial No. NDS(I)-72 LPP 1956
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Physiology and neuropathology of acute total asphyxia in the rhesus monkey

During FY 1973 this project was incorporated with Serial No. NDS(I)-73 LPP 2020.

Serial No. NDS(I)-72 LPP 1957
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Methods of quantification of myocardial contractility in the rhesus monkey

Previous Serial Number: Same

Principal Investigators: David M. Mirvis, M.D.
Gary S. Kopf, M.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:
Total: 1.85
Professional: 1.07
Other: .78

Project Description: Project completed

Honors and Awards: None

Publications:

Mirvis, D. M., Kopf, G. S., and Pottala, E.: The effect of low frequency response catheter systems on the calculation of force velocity parameters in the intact heart. Proc. Rocky Mountain Bioengineering Symposium In press.

Serial No. NDS(I)-72 LPP 1958
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neuropathology and physiological response of rhesus monkeys to hypoxia on heart-lung bypass

During FY 1973 this project was incorporated with Serial No. NDS(I)-73 LPP 2020.

Serial No. NDS(I)-72 LPP 1959
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Physiology and neuropathology of profound hypoxia in the rhesus monkey

During FY 1973 this project was incorporated with Serial No. NDS(I)-73 LPP 2020.

Serial No. NDS(I)-73 LPP 2020
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Pathophysiology of brain injury produced by cardiovascular and pulmonary disorders

Previous Serial Number: None and incorporating Serial Nos. NDS(I)-69 LPP 1715, NDS(I)-71 LPP 1901, NDS(I)-71 LPP 1902, NDS(I)-72 LPP 1956, NDS(I)-72 LPP 1958, and NDS(I)-72 LPP 1959

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Harvey M. Shapiro, M.D.
Michael J. Labance, M.D.
Dennis J. Selkoe, M.D.
David M. Mirvis, M.D.
Gary S. Kopf, M.D.
Francis W. Gamache, M.D.

Cooperating Units: CR:LAS

Man Years:

| | |
|---|---|
| Total: | 2.85 |
| Professional: | 2.07 |
| Other: | .78 |

Project Description:

Objectives: To develop animal experimental models of human brain disease produced by a variety of cardiovascular and pulmonary disorders. These include studies where there are interferences with oxygen availability to the tissues, either due to cessation of circulation (circulatory arrest), hypotension, pulmonary disease, biocardial abnormalities, intoxications with such agents as carbon monoxide, cyanide, or, finally, hypoglycemia.

Methods Employed: In general, juvenile or adult monkeys are anesthetized with pentobarbital, their femoral arteries and femoral veins catheterized for recording of blood pressure, heart rate, and for the withdrawal of blood samples for analysis of acid-base and respiratory gas state. Electrocardiographic and electroencephalographic leads are placed on the animal and a variety of other special methods are employed, such as the measurement of cerebral impedance, intracranial pressure, withdrawal of samples of cerebral spinal fluid from various sources, etc., are employed where necessary to evaluate specific physiological or biochemical changes. The animals are subjected to a variety of insults which include circulatory arrest, hypotension, hypoglycemia, ventilation with atmospheres low in oxygen, etc. Measures are taken of the variety of physiological and biochemical changes which are produced by these episodes of insult.

Major Findings: In the findings obtained thus far, a variety of new insights have been achieved. For example, the neuropathology of carefully regulated episodes of total circulatory arrest has been defined as one which effects structures located in the brain stem. Hemispheral structures, such as cortex white matter and basal ganglia, remain unaffected. These findings are quite contrary to those which have been classically described as occurring in the human as the pathology of circulatory arrest. It now becomes clear that the human patterns of injury following circulatory arrest relate not to the actual stasis of blood flow but to difficulties incurred in relation to resuscitation during which severe degrees of partial asphyxia are sustained by the human. Another reason for failure of the lower brain stem pattern of injury in the human to occur is the involvement of the heart in the human clinical situation with reference to circulatory arrest and a consequent myocardial injury which limits survival when the episode extends beyond 5-8 minutes. Thus, the pattern of circulatory arrest is closely similar to the pattern of total asphyxia and, in both instances, the brain tissue is subjected to a total absence of availability of oxygen throughout much of the episode.

The severity of hypotension which the primate might sustain without evidence of brain injury has been found to be in the order of magnitude of 30 mm Hg average arterial blood pressure. In general, animals subjected to such severe degrees of hypotension either manifest an intact survival afterwards or will die during the period of hypotension, usually with cardiac rhythm abnormalities; they may die in the early hours following the restoration of blood volume of intractable hypotension and circulatory failure; or, finally, they may die during the first 24-48 hours with severe brain swelling and brain compression. Only very small numbers of animals subjected to severe hypoglycemia have survived to show static, long-term patterns of pathology.

Hypoglycemia has been shown to be associated with relatively minor alterations in respiratory gas and acid-base state in the blood but to be associated with degrees of hypotension and circulatory impairment. When the animals are artificially respired, the patterns of pathology observed involve the basal ganglia in foci of acute necrosis, and the cortex with superficial layers of necrosis and associated dropout of neurons. Over a long period of time, the latter changes are associated with convolutional atrophy and dementia.

Significance: These studies with energy deprivation states of the nervous system associated with cardiovascular or pulmonary diseases are important to us for the elucidation of the pathophysiology and the pathogenesis of important patterns of brain injury in the human.

Proposed Course of Project: These studies will be continued to attempt further clarifications of the physiological changes and their relationship to the patterns of pathology generated.

Honors and Awards: None

Publications:

Miller, J. R., and Myers, R. E.: Neuropathology of systemic circulatory arrest in adult monkeys. Neurology 22: 888-904, 1972.

Selzer, M. E., Myers, R. E., and Holstein, S. B.: Unilateral asphyxial brain damage produced by venous perfusion of one carotid artery. Neurology 23: 150-158, 1973.

Serial No. NDS(I)-73 LPP 2021
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Short- and long-term behavioral deficits produced by dysergic brain disease

Previous Serial Number: None

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Shun-ichi Yamaguchi, Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.01 |
| Professional: | .40 |
| Other: | .61 |

Project Description:

Objectives: To determine the short- and long-term effects on perceptual learning and memory function of a variety of insults based on cardiovascular and pulmonary disease and/or states of intoxication.

Methods Employed: Juvenile rhesus monkeys are trained on visual pattern discrimination tasks to choose one of two visual stimuli and to avoid the other. Banana pellet food reward will be used to reinforce the correct pattern of responding. The monkeys are then subjected to episodes of energy deprivation (such as circulatory arrest, severe hypotension, severe hypoxia, total asphyxia, carbon monoxide, etc). The animals are then resuscitated and subjected to testing on the behavioral apparatus to determine effects on performance levels and to determine the time course of restoration of correct performance. Permanent deficits will be determined and quantitated.

Major Findings: This project is just being initiated and thus far no findings are available.

Significance: The neurological assessment of the clinical state of the rhesus monkey is difficult and leads to only rather crude knowledge as to the neurological implications of specific insults. Utilization of the present sophisticated behavioral test methods should permit the assessment of subtle changes in overall nervous system activity, both in terms of temporary reversible metabolic disturbances and also in relation to permanent, long-lasting deficits related to specific lesions.

Proposed Course of Project: To proceed with specifically designed experiments according to the general objectives stated.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LPP 2022
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neural mechanisms underlying social behavior and emotion in the rhesus monkey

Previous Serial Number: None and incorporating Serial No. NDS(I)-70 LPP 1824

Principal Investigator: Shun-ichi Yamaguchi, Ph.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 1.01 |
| Professional: | .40 |
| Other: | .61 |

Project Description:

Objectives: To investigate the neuromechanisms underlying social behavior and emotion in the rhesus monkey.

Methods Employed: A variety of techniques are used in order to study the social behavior and emotion in the sub-human primate. In some instances, monkeys are placed in reaction chambers and their rates of generation of spontaneous patterns of vocalization are determined. In other instances, the animals are observed for their cage behavior and their reaction to challenge by the experimentor. In other instances, where possible, animals are placed in small gang cages as small social groups, and their interactions measured.

Major Findings: Studies thus far have demonstrated that lesions of cortical areas related to speech in humans failed to alter vocalization in the rhesus monkey, while areas of cortex having to do with the regulation and control of social behavior, which includes the prefrontal and anterior temporal cortex, produce major disturbances or total abolition of vocal response permanently in the rhesus monkey. Studies of subcortical foci are currently in progress and, thus far, it has been determined that amygdala and tegmentum of brain stem, both lower centers for regulation of social behavior, show major effects on patterns of vocal response. Studies in gang cages have demonstrated major deficits in all aspects of social interaction and social reaction with lesions of prefrontal and anterior temporal cortex. Lesions of cingulate cortex have much lesser effects. Other studies with animals having crossing retinal fibers at optic chiasma transected have shown that, in general, unilateral lesions of prefrontal and/or of anterior temporal cortex have bilateral implications as tested over the two sides of the body. When second-sided lesions are added to first-sided lesions, then again further bilateral defects are engendered. Thus, there exists both elements of laterality and of bilaterality of these functions of the cortical mechanisms regulating social behavior. Further studies are trying to determine the pathways utilized for the bilateral effects exerted by unilateral lesions and the sparing functions thereof.

Significance: These studies which attempt to identify those brain structrues involved in regulation and control of social behavior are of major importance to our efforts to understand the biological foundation of violence and other disorders of behavior which may be observed as part of our modern day life.

Proposed Course of Project: To pursue and conduct specific experiments according to the general objectives stated.

Honors and Awards: None

Publications:

Hara, K., and Myers, R. E.: Role of forebrain structures in emotional expression in opossum. Brain Res. 52: 131-144, 1973.

ANNUAL REPORT
July 1, 1972 through June 30, 1973
Laboratory of Molecular Biology
National Institute of Neurological Diseases and Stroke
Intramural Research

Ernst Freese, Chief

The work of the laboratory has been concerned with three different but related topics, the control of nucleic acid synthesis and differentiation in bacteria, the transport of substrates and its inhibition in bacteria and human cells, and the control of mammalian virus replication. In comparison to higher organisms, the work with bacteria proceeds faster, allows more reliable interpretations, and permits the use of powerful genetic methods; it has yielded a host of new results which should be applicable to mammalian cells, since their nucleic acids and membranes, as well as their general metabolic pathways, have basic properties similar to those of bacteria. For example, studies on isolated bacterial membranes have shown that, in contrast to previous assumptions, the transport of most molecules does not require ATP but is energized by other high energy compounds or by the electron transport system in the absence of ATP. Similar studies in human tissue cultures have now also demonstrated that ATP is not required for most substrate transport. Furthermore, the transport of amino acids into bacteria or their membrane vesicles can be inhibited by lipophilic acids, including most presently used antimicrobial food additives. Recent studies have shown that the same compounds inhibit tissue cultures of various human cells at least as much. While the inhibition of human cells had been predicted from the bacterial studies, two additional findings were surprising: 1) Certain viruses could not grow in human cells when fatty acids were present. 2) In the presence of short chain fatty acids or parabens epithelial-like cells developed a jagged morphology with some cells containing long spikes. These compounds did not affect fibroblasts, whose morphology in turn is specifically altered by butyryl-cyclic AMP. Another set of studies was concerned with the control of RNA and DNA synthesis in bacterial cells whose permeabilization by toluene or cold shock enabled the study of nucleic acid synthesis without disrupting the three-dimensional relationship between enzyme and membrane components. In particular, the inhibition of RNA synthesis by guanosine tetraphosphate (ppGpp), discovered in this laboratory, could thus be studied. These examinations were greatly helped by the isolation of mutants lacking certain components of the DNA or RNA synthetic machinery. Similar studies examined the RNA synthesis of mammalian viruses, such as the vesicular stomatitis virus, which represent a group of virus diseases that produce various neurological defects. It was found that the complementary RNA synthesized by the virus contains long stretches of polyadenylic acid against which specific chemotherapeutic agents might be developed. This finding and the inhibition of virus replication by small concentrations of lipophilic acids indicate that various means of controlling certain virus diseases may become available. Finally, the examination of differentiation in normal and cacogenic mutant cells of bacteria may provide clues to the treatment of certain hereditary human diseases. It was shown by mutants lacking the first gluconeogenic enzyme that gluconeogenesis is required for differentiation, but normal differentiation could be restored by the frequent addition of small amounts of gluconate. Mutants in which glucose-6-phosphate or glycerol phosphate accumulate could not differentiate normally, but means have been found to prevent this accumulation and thus to restore differentiation. Furthermore, mutants unable to produce glycerol phosphate and thus membrane lipids required glycerol for growth but could differentiate only when the medium also contained malate. These results will now be described in more detail.

## 1. Membrane Transport in Bacteria and Human Cells

The analysis of membrane transport has been greatly advanced by the finding that membrane vesicles can be isolated which are essentially free of cytoplasm and for which transport depends on the addition of external energy sources. In particular, the transport of amino and keto acids depends on the addition of compounds that can be oxidized by the electron transport system of the cell, e.g., NADH, glycerol phosphate, or reduced phenazine methosulfate. Previous experiments in this laboratory have found that NADH is much less efficient, per molecule oxidized, in transporting amino acids than is glycerol phosphate. This phenomenon has been attributed to either inefficient coupling of NADH oxidation to amino acid transport or to the existence of different electron transport pathways. However, recent experiments have shown that these differences in fact result from the presence of open or inverted vesicles in the membrane preparations. In such vesicles the NADH oxidation activity is directly available to external NADH which can be rapidly oxidized without leading to any amino acid transport. In contrast, in closed vesicles in which the normal side looks to the outside, the transport of NADH is the rate-limiting step, allowing a reasonably efficient energization of amino acid transport only at high NADH concentrations. In intact cells the NADH oxidation is also completely located on the inside and therefore only very slowly accessible to external NADH. However, when these cells are lysed, the rate of NADH oxidation increases about 20-fold. In contrast, the rate-limiting reaction of glycerol phosphate oxidation is that of glycerol phosphate dehydrogenase, so that closed or open vesicles oxidize glycerol phosphate at essentially the same rate. It is therefore concluded that both NADH and glycerol phosphate energize amino acid uptake only from within the membrane vesicle and that the lower efficiency of NADH is caused by its low entry rate into the membrane.

All inhibitors of the electron transport system inhibited oxygen consumption and amino acid transport in both whole cells and membrane vesicles. However, rotenone partially inhibited NADH oxidation in vesicles, whereas it had no effect on amino acid transport. In conjunction with the above experiments it was concluded that rotenone was unable to enter the membrane and inhibited only the NADH oxidation activity of open or inverted vesicles.

Other compounds inhibiting amino acid transport were the fatty acids or DNP; they apparently uncouple the amino acid transport from the electron transport system, because they did not inhibit the oxidation of NADH or glycerol phosphate by membrane preparations. The effect of the fatty acids increased with decreasing pH and increasing chain lengths, indicating that the unionized acid inhibits by attaching to the membrane. The short chain fatty acids up to hexanoic acid inhibited Gram-positive and Gram-negative bacteria equally. However, the medium or long chain fatty acids inhibited only Gram-positive bacteria, whereas they had no effect on the Gram-negative ones. This phenomenon can be used to distinguish between Gram-positive and Gram-negative organisms. The resistance did not result from a resistant set of transport proteins, because the transport into membrane vesicles of E. coli was inhibited by long chain fatty acids. Various mutants of E. coli and S. typhimurium, both Gram-negative organisms, were therefore investigated. The ability of the normal Gram-negative strains to metabolize long chain fatty acids was not responsible for their resistance, because mutants deficient in this metabolism were still resistant. However, mutants of the Gram-negative organisms partially lacking the lipopolysaccharide layer (stripped from the KDO core out) were sensitive to inhibition by medium chain fatty acids such as decanoate, with respect to both growth and amino acid transport. The differential inhibition by decanoate can be used to distinguish between normal and mutant Gram-negative organisms. It shows that the openings of the lipopolysaccharide layer are normally too small to permit the entry of longer fatty acids. Also, when the lipopolysaccharide layer was partially stripped by EDTA treatment did the Gram-negative organisms become transiently sensitive to long chain fatty acids; but they returned to their resistant state within 30 minutes of metabolism during which the layer apparently could be repaired. Since both Gram-positive and Gram-negative bacteria are resistant to small lipophilic acids, these compounds may be useful to combat infection by such organisms.

To compare the transport in bacteria with that in mammalian systems, the uptake of amino acids was measured in tissue cultures of human fibroblasts. Similar to results originally described by Christensen for tissue slices, fibroblasts contain the "A" and "L" transport systems for different amino acids, the A system being dependent on sodium, whereas the L system is not. It was shown that the transport of alanine by the sodium-dependent system continued even when the ATP levels had fallen 1,000-fold in the presence of inhibitors of the electron transport system, indicating that ATP is not necessary for the sodium-activated transport. The requirement for sodium was specific, since lithium, potassium, ammonium, Tris, or choline were ineffective. At low sodium concentrations, however, inhibitors of the electron transport system or of the sodium-potassium ATPase inhibited amino acid transport, presumably because at this level of external sodium the sodium gradient could be maintained only in the presence of an active Na-K-ATPase. Fatty acids also caused an inhibition of transport, but only if the cells were preincubated in their presence for at least 6 minutes. The reasons for this delayed effect, which can nevertheless be responsible for the long-term inhibition of growth, is under investigation.

### 2. Effect of Antimicrobial Food Additives on Bacteria and Human Cells

Most antimicrobial food additives are lipophilic acids which inhibit the growth of bacteria. Since Gram-negative organisms had shown a protection against long chain fatty acids, the effect of food additives on Gram-positive and Gram-negative organisms was compared. All generally used antimicrobial food additives that have been used for many years inhibited both organisms, indicating that they were small enough to pass through the pores of the lipopolysaccharide layer in Gram-negative bacteria. However, some of the more recently invented food additives containing long aliphatic chains should be able to pass this layer.

Since the antimicrobial food additives affect cell membranes, it appeared likely that they would also affect the membranes of human cells. In fact, it was observed that all these compounds inhibit the growth of human epithelial-like or fibroblast cells at least as efficiently as that of microorganisms. These results show that too extensive use of such compounds may be harmful to the function of some human cells. In fact, intestinal cells probably are not seriously exposed to the lipophilic acid ingested with food only because these compounds are rapidly removed into the blood stream by an ion-concentrating mechanism which depends on the low pH in the gut and the neutral pH in the surrounding tissues; they can subsequently be detoxified because they are either degraded or used he synthesis of other compounds.

Apart from inhibiting growth, the smaller lipophilic acids oduce pronounced morphological changes in HeLa and other ithelial-like cells but not in fibroblasts. For example, en HeLa cells were incubated in the presence of 5 mM butyrate, lamentous protrusions appeared 7-8 hours later and developed to long spikes by 24 hours. Propionate, hexanoate, methyl- or yl-parabens produced the same morphological changes as buty- te. Under these conditions the cell viability did not signif- antly decrease and the cell shape returned to normal as soon the inhibitor was removed. Addition of cAMP or butyryl-cAMP ld not cause such alterations; these compounds cause similar terations in fibroblasts which are not affected by the fatty ids. The mechanism causing these shape changes is under inves- gation.

### 3. Control of Mammalian Virus Replication

A number of myxo-, paramyxo-, and rhabdo-viruses contain A which replicates by means of an RNA-dependent RNA polymerase ntained in the virion itself. Such viruses cause a variety of seases in which nerves and in particular the central nervous stem are affected. Examples are mumps, measles, canine dis- nper, Newcastle's disease, encephalitis, or meningitis. Two ruses of this group that cause no or only mild human symptoms been used for experimental study, the vesicular stomatitis rus (VSV) and the Sindbis virus. The VSV virus was found to ntain 7 proteins, 5 of which can be seen in SDS polyacrylamide l electrophoresis, while two, a protein kinase and a nucleo- de diphosphokinase, could be detected only by enzyme assays. RNA polymerase, contained in the virus core, produced during transcription of the virus RNA long polyadenylic tracts, as shown by chromatography on oligo-dT-cellulose columns. Since is RNA completely hybridized with the virus RNA, it reflects curately the early RNA synthesis of the infected cell. The g stretch of adenylic residues renders this RNA particularly nsitive to potential chemotherapeutic agents for which the veloped assay provides a simple screen. This lead will now be ther investigated.

In conjunction with the investigation of food additives, it found that the capacity of HeLa cells to support the growth Sindbis or VSV was greatly reduced by an exposure to butyrate, n did not affect the growth of other riboviruses such as io. In the butyrate-treated cells little or no virus-specific was synthesized, whereas the absorption, penetration, uncod- , or maturation of the Sindbis virus were unaffected. This licates that butyrate may rather specifically inhibit the RNA lication of certain viruses.

### 4. Control of RNA and DNA Synthesis

The cellular synthesis of DNA and RNA is subject to a number of control mechanisms, most of which are still not understood. Since it has not yet been possible to exhibit the known control mechanisms in vitro, the notion has developed that the arrangement of cellular components and their attachment to membranes may be essential to maintain the normal cellular control mechanisms. One would therefore like to have conditions under which the cellular structure is altered as little as possible. Since the cellular membrane normally does not permit the entry of the direct precursors of nucleic acid synthesis, the best approach uses cells that have been permeabilized to the entry of such nucleotide triphosphates by as mild methods as possible. Two such methods have been used and have already yielded differences that point to a very delicate control of DNA synthesis via the cellular membrane. When cells were permeabilized by exposure to toluene, both the normal replication of DNA and repair synthesis could subsequently be observed. In contrast, when these cells were permeabilized by sudden exposure of the cells to a low temperature, only repair synthesis could be observed, as DNA replication was absent. This conclusion could be drawn because a particular inhibitor, 6-(p-hydroxyphenylazo)-uracil, inhibits DNA replication (i.e., DNA polymerase III) and not DNA repair. The conclusion could also be verified by the use of Bacillus subtilis mutants that are defective in repair synthesis, because they lack DNA polymerase I. The reasons for this difference in the two permeabilization procedures will have to be worked out. The cold-shock treatment showed the other surprising property that it released the cellular UV-absorbing material only if the cells were exposed to a temperature below 7°. This could mean either that the cellular membrane stiffens at this transition temperature or that water clathrates rupturing the membrane can form only below this temperature. Since the storage of cells, including blood, transplantation tissues, and tissue cultures, is very important, this phenomenon will be analyzed in detail.

The permeabilization of Escherichia coli has been used to examine the control of ribosomal RNA synthesis. In intact cells this synthesis is stopped by amino acid starvation or by reduced nutrient supply (stepdown); under these conditions a compound, ppGpp, isolated in the laboratory, increases greatly in amount. When ppGpp was added to permeabilized cells, the total RNA synthesis was reduced by 50%, but a specific inhibition of ribosomal RNA synthesis was not observed. Apparently, ppGpp alone does not directly inhibit rRNA synthesis, but it may do so via some factor that is missing in the permeabilized cell system.

In order to examine the role of ppGpp further, a mutant was isolated in which this compound still accumulated upon amino acid starvation but decreased at a 20-fold reduced rate when the missing amino acid was readded. Under the same conditions under which ppGpp was not degraded, the strain did also not recover RNA synthesis, indicating that ppGpp is somehow involved in this recovery. The metabolism of ppGpp was completely inhibited when the antibiotic oxytetracycline was added; under these conditions RNA synthesis could not recover even in complete medium containing all known growth factors. Since oxytetracycline is a known inhibitor of protein synthesis, it appears likely that ribosomes or the protein synthetic apparatus of the cell is involved in both the synthesis and the further metabolism of ppGpp. Another mutant isolated could not synthesize RNA at elevated temperatures. However, after permeabilization both mRNA and rRNA were synthesized at the same rate as in the parent strain; the mutant apparently accumulated at the elevated temperature some inhibitor of RNA synthesis which could diffuse out of the cell upon permeabilization.

### 5. Defective Differentiation and its Cure in Cacogenic Mutants

The process of differentiation is under the delicate control of many genes, most of which are not essential for the vegetative replication of cells but exert their critical role only during the developmental process. Cacogenic mutants, in which genes are defective that are necessary only for differentiation, can be found in both microorganisms and higher organisms. In humans such deficiencies lead to abortion, or if they survive, to various hereditary diseases. To understand their role in differentiation and to obtain possible cures of such deficiencies, a number of cacogenic mutants have been isolated in Bacillus subtilis, and their effect on sporulation has been studied. For example, to examine the control of gluconeogenesis in differentiation, the $CO_2$-fixing and liberating enzymes have been examined. The constitutive enzyme pyruvate carboxylase, which was strongly activated by acetyl Co-A, was necessary for growth on glucose as shown by a mutant. However, the enzyme was not required for sporulation in a complex medium. In contrast, the first gluconeogenic enzyme, phosphoenolpyruvate carboxykinase was repressed by glucose and not necessary for growth, but it was required for sporulation. However, mutants lacking this enzyme could sporulate normally when small amounts of gluconate were frequently fed during the developmental period.

Since glucose effectively suppresses sporulation, mutants were obtained in which glucose-6-phosphate could not be metabolized into any other compound. Such mutants sporulated normally when they were grown in a rich medium not containing glucose, but the addition of the smallest amount of glucose completely suppressed sporulation. The cells of another mutant, in which glucose-6-phosphate could be converted into glucose-1-phosphate and the sugar nucleotides, grew to a low titer and then lysed extensively. This lysis could also be induced by growth in the presence of galactose. Evidence collected by a variety of additional mutants indicated that the deficiency was caused by the accumulation of some sugar nucleotide in the cell. The phenomenon resembles that observed in galactocemia. The mechanism by which accumulation of these sugar nucleotides induces cell lysis and lack of differentiation is under investigation.

Another extremely interesting set of mutants was isolated in which glycerol phosphate can either not be made from or degraded into components of the Emden-Meyerhof path. The mutants in which glycerol phosphate cannot be degraded accumulated this compound, were unable to differentiate, and showed under the electron microscope asymmetric membranes that were filled with cell wall material instead of being thin as required for normal sporulation. The cell repeatedly formed new asymmetric septa but each in turn was filled with cell wall material, thus preventing the further membrane invagination and spore development. In the presence of malate, however, these mutants were completely cured, because malate repressed the formation of glycerokinase and thus the accumulation of glycerol phosphate. The mechanism of this repression is still under investigation. The other mutants, unable to produce glycerol phosphate by de novo synthesis, were also unable to sporulate, except if glycerol was added continuously throughout the developmental period in very small quantities; larger glycerol quantities suppressed sporulation. As another way of insuring the supply of small quantities of glycerol phosphate, the cells could be grown in the presence of both glycerol and malate, in which case malate again assured a slow supply of glycerol phosphate.

Serial No. NDS(I)-65 LMB/DB 1244
1. Lab. of Molecular Biology
2. Section on Developmental Biology
3. Bethesda, Maryland

Individual Project Report
PHS-NIH
July 1, 1972 through June 30, 1973

Project Title: Control Mechanisms and Differentiation

Previous Serial Number: SAME

Principal Investigator: Ernst Freese, Ph.D.

Other Investigators: Martin D. Diesterhaft, Ph.D., Richard C. Henneberry, Ph.D., Chandan Prasad, Ph.D. (Visiting Fellow)

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 4.8 |
| Professional: | 4.2 |
| Other: | 0.6 |

Project Description:

Objectives: The process of differentiation, which begins when cells stop growing and then undergo morphological changes, is under the control of regulatory mechanisms, the essence of which should be similar in micro- and macroorganisms. Microorganisms can faster provide a detailed model of differentiation, because they multiply rapidly, allow the isolation of (cacogenic) mutants affecting differentiation and their genetic analysis, and permit the production of large quantities of differentiating organisms, all at the same developmental stage, for biochemical studies. Our work has concentrated on reactions that are likely to be important for the control of differentiation in all organisms, such as the relevance of gluconeogenesis, the suppression of differentiation by different compounds in mutants that are representative of hereditary diseases, and the control of enzyme and DNA synthesis.

Methods Employed: Following the isolation of mutants, specific strains containing certain combinations of mutations were obtained by genetic transformation with DNA and selection of the desired mutants on specific media or by resistance to phage SP8.

For the assay of PEP-carboxykinase, a specific and sensitive assay was developed in which the $^{32}P$ of $\gamma$-$^{32}P$-ATP is converted to PEP, cleaved by manganese, and then assayed after charcoal absorption of the remaining ATP.

For the permeabilization of cells a technique was developed in which they are exposed to low temperatures (e.g., 0°) for 5 min and then collected by centrifugation. To separate DNA synthesis into DNA replication and repair, UV and MMS-resistant mutants lacking repair were used and the specific inhibitor of DNA replication, 6-(p-hydroxyphenylazo)-uracil was employed.

Major Findings: 1. Control of gluconeogenesis and its relevance for differentiation. $CO_2$ fixation in Bacillus subtilis was found to occur primarily through the apparently constitutive enzyme, pyruvate carboxylase, which is strongly activated by acetyl-CoA. This enzyme is necessary for growth on glucose but not required for sporulation, as was established with a pyruvate carbosylase mutant. The reverse production of pyruvate from citric acid cycle compounds is catalyzed by the malic enzyme which uses either NAD or, less effectively, NADP as cofactor; the ratio of these activities remains constant through enzyme purification and during the induction of the enzyme's activity by growth in the presence of malate; the enzyme synthesis is not repressed by glucose. Malic enzyme and pyruvate carboxylase form a "pyruvate shunt" of the citric acid cycle, which apparently is necessary for growth on malate or for the production of oxaloacetate from malate in substrate amounts; malic dehydrogenase functions mainly to provide energy.

The first gluconeogenic enzyme in PEP-carboxykinase, using a specific and sensitive assay PEP-carboxykinase, was purified and showed a $K_m$ for oxaloacetate of about 25 uM about a factor of 10 lower than previous estimates. Enzyme synthesis is repressed by glucose but relieved during sporulation. PEP-carboxykinase-deficient mutants have established that the enzyme is needed for both gluconeogenesis and for sporulation. However, sporulation of such mutants could be restored by the continued addition of gluconate. This is another case of the increasing number of cacogenic mutants which have been isolated in our laboratory and in which sporulation could be restored to almost th normal frequency; it shows how the feeding or witholding of specific compounds can allow normal development in spite of hereditary defects.

2. Examination of a pleiotrophic mutant unable to differentiate. During the examination of cacogenic sporulation mutants a particularly interesting strain was found in which several enzymes must be affected. The mutant produces only one

of the two aspartokinase activities, with the result that its growth is inhibited by lysine but restored by threonine. It cannot produce any gluconate kinase, but it produces constitutively a glucose dehydrogenase which normally is made only during the sporulation process and for which no control mechanism has yet been found. Surprisingly, dialysis of the mutant extract revealed the missing (diaminopimelate inhibitable) aspartokinase activity but with different $K_m$ value; the enzyme obviously was inhibited by some cell component by which the normal enzyme is not inhibited. The genetic and biochemical nature of this pleiotrophic mutant effect, which is not related to any known suppressor mutation, is under investigation.

3. Suppression of sporulation and control of cell wall properties by sugar derivatives. By the use of phage resistance and other genetic techniques, double and triple mutants have been isolated in which glucose-6-phosphate can either be converted only into sugar nucleotides or cannot be metabolized at all. It was found that a double mutant lacking both phosphoglucose isomerase and glucose-6-phosphate dehydrogenase activities grew only to a low turbidity and then started to lyse. When a third mutation was induced in phosphoglucomutase, the lysis did not occur, but when such a third mutation was introduced in teichoic acid transferase, the same lysis as before was observed. Lysis could be prevented by any compound that could enter the glucosamine path in the mutant. This indicates that some compound in the sugar nucleotide metabolism was responsible for lysis, similar to the phenomenon in which the accumulation of sugar nucleotides is responsible for galactocemia. In fact, the feeding of galactose to a mutant not susceptible to lysis with glucose still induced the lysis. However, additional mutations were isolated in which this galactose-induced lysis was prevented. Surprisingly, all known galactose and glucose nucleotide enzymes were found to be present in these galactose-resistant mutants, which shows that some unknown enzyme or control mechanism must normally be present which is defective in these mutants. This phenomenon is still under investigation.

4. Control of DNA synthesis in cells permeabilized by cold treatment. In order to study the capacity of cells to synthesize DNA at different stages of development, we have developed a technique that renders cells of Bacillus subtilis permeable to phosphorylated nucleotides without being lethal. Exposure of cells to a brief cold shock causes these permeability changes, which can be monitored by release of material absorbing at 260 nm. The resulting cells incorporate labeled deoxyribonucleotide triphosphates into DNA by a reaction which is insensitive to 6-(p-hydroxyphenylazo)-uracil, a known inhibitor of DNA replication. The incorporation also does not occur in several DNA repair mutants defective in polymerase 1. Incorporation of

the nucleotides apparently reflects DNA repair rather than replication. When cells were treated with toluene rather than with cold shock, they were also permeabilized but normal replication, inhibitable by 6-(p-hydroxyphenylazo)-uracil, occurred in the presence of the deoxynucleotide triphosphates. In order to understand the difference of the permeabilization processes, the temperature required for the cold shock was examined. Surprisingly, it was found to be below 7° with a rather abrupt temperature dependence. We are investigating at present two possibilities to explain this temperature sensitivity. One is that this membrane has a transition temperature to become stiff within thi temperature range. An intriguing alternative explanation is tha the permeability changes reflect water clathrate formation on or in the cell membrane. Our system may therefore provide a very simple tool to measure the production of such clathrates, which have been proposed as an explanation for anesthetics.

Significance to Biomedical Research and the Program of the Institute: The mutants examined in this work are blocked in som of the same enzymes whose deficiency in human hereditary disease produce galactocemia or hemolytic anemia. The accumulation of sugar phosphates and nucleotides may also be responsible for teratogenic effects. Whereas it is difficult in higher organism to determine why these compounds produce diseases or abnormalities, the isolation of multiple mutants in the bacterial system allows one to uncover these causes. In addition, specific techniques can be detected by which the normal development of bacterial mutants can be restored; a similar treatment could then be attempted for mammalian diseases. The analysis of cold-shocked cells promises to be important for the storage of blood and tissue cultures, because similar mechanisms releasing the cell content at temperatures above 0° should also operate on other cells, if they are associated with properties of all cell membranes. So far only freezing of cells has been extensively examined by others.

Proposed Course of Project: Mutants will be further investigated that accumulate sugar phosphates and sugar nucleotides, and as a consequence either lyse or are unable to sporulate. Attempts will be made to find simple techniques to phenotypicall cure the mutants and to determine why the accumulation of the above compounds prevents their normal development. The mechanis by which cold treatment causes the opening of cells and the release of their content will be further investigated. These studies will be compared with the clathrate formation caused by anesthetics or hypothermia and with the low temperature exposure of blood or tissue cultures.

Honors and Awards: None

Publications:

Diesterhaft, M. D. and Freese, E.: Pyruvate kinase of *Bacillus subtilis*. *Biochim. Biophys. Acta* 268: 373-380, 1972.

Freese, E.: Sporulation of Bacilli, a model of cellular differentiation. In Moscona, A. A. and Monroy, A. (eds.): *Current Topics in Developmental Biology*. New York, Academic Press, 1972, Vol. 7, pp. 85-125.

Rhaese, H. J. and Boetker, N. K.: The molecular basis of mutagenesis by methyl and ethyl methanesulfonates. *Eur. J. Biochem*. 32: 166-172, 1973.

Diesterhaft, M. D. and Freese, E.: Role of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and malic enzyme during growth and sporulation of *Bacillus subtilis*. *J. Biol. Chem.*, in press.

Serial No. NDS(I)-71 LMB/DB 1886
1. Lab. of Molecular Biology
2. Section on Developmental Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Developmental Cytology

Previous Serial Number: SAME

Principal Investigator: Elisabeth B. Freese, Ph.D.

Other Investigator: Yong K. Oh, Ph.D. (Visiting Fellow)

Cooperating Units: None

Man Years:

| | |
|---|---|
| Total: | 3.0 |
| Professional: | 2.0 |
| Other: | 1.0 |

Project Description

Objectives: Differentiation consists of a sequential development leading from vegetatively-growing cells to highly differentiated ones. We want to correlate changes of the morphological fine structure and of enzyme alterations of samples at the different times and thus find out which biochemical reactions are needed for each developmental step. Such an analysis can be performed using sporulation of Bacillus subtilis, an organism whose cells grow rapidly, can be genetically transformed, and for which mutants can be easily isolated. A correlation between structural and biochemical events is particularly feasible in developmental mutants blocked or artificially arrested at certain stages of development, or in mutants able to differentiate under some conditions but not under others (e.g., mutants in the glycerol or glucose metabolism).

Methods Employed: For the morphological analysis, ultrathin sections were studied with the electron microscope EM6B of Associated Electrical Industries. The standard strain and developmental mutants of Bacillus subtilis were grown to different stages of development, samples were centrifuged, fixed by glutaraldehyde, stained with osmium tetroxide and uranyl acetate, dehydrated, embedded in Epon, and after sectioning with the Reichert microtome OmU2, stained by lead citrate. Developmental mutants were induced by ethylmethanesulfonate or γ-irradiation. They were plated out on a mixture of carbon sources and then replicated to plates with single carbon sources. Non-growth on one or more of these carbon sources gave indications as to where the biochemical block must be. This technique can be used to select specific mutants. Double and triple mutants were constructed either by transformation of a single or double mutant with the DNA of another mutant, or by virus mediated transductio

The accumulation of metabolites in mutants was determined, e.g., for mutants in the citric acid cycle, by the separation of radioactive compounds on thin-layer plates with cellulose. Glycerol phosphate accumulation was measured spectrophotometrically using glycerol phosphate dehydrogenase. The NAD-independent and the NAD-dependent glycerol phosphate dehydrogenase and the glycerol kinase activities were assayed according to Lin et al. (*Proc. Natl. Acad. Sci. USA* 48: 2145-2150, 1962).

Major Findings: 1. Suppression of sporulation in citric acid cycle mutants. a) Mutants blocked in different enzymes of the citric acid cycle have been isolated in this laboratory or were obtained from Drs. Hanson (University of Wisconsin) and Hoc (University of California). They did not sporulate normally, an the addition of glutamate, gluconate, or malate did not restore sporulation. The production of ATP in these mutants stops early in the developmental period. Extensive testing showed that the mutants could be subdivided into two classes with respect to their sporulation ability. Mutants of the first half of the citric acid cycle lacking aconitase, isocitrate dehydrogenase or -ketoglutarate dehydrogenase grew to a low optical density and produced essentially no spores. In contrast, mutants in the second half of the cycle lacking succinic dehydrogenase, fumaras or malic dehydrogenase produced some spores, from $10^4$-$10^6$ spores/ml. Under the same conditions the standard *B. subtilis* strain produces $10^8$-$10^9$ spores/ml.

b) Electron microscopic investigations revealed that the majority of the mutant cells were blocked very early in differentiation, before the asymmetric membrane developed. Aconitase an isocitrate dehydrogenase mutants stopped their development befor the axial filament stage, but they could proceed to this stage i the medium contained additional glutamate, which the mutants are unable to synthesize. The formation of axial DNA filaments that are stretched from one pole of the cell to the other represent the first clear sign of differentiation. All other citric acid cycle mutants proceed to this stage without any additions. The next stage of sporulation, the asymmetric membrane septation, wa not observed during the screening of 100-1,000 cells for each mutant.

c) To test whether the bipartition of citric acid cycle mutants according to sporulation frequency was correlated to the accumulation of a common metabolic intermediate, the dissimilation of both $^{14}$C-malate and glutamate was measured. While the metabolic intermediates within each half of the cycle can flow in both directions, there is only a one-way flow possible between the two halves, from α-ketoglutarate to succinyl-CoA and from malate to citrate. We found that the aconitase and isocitric dehydrogenase mutants accumulated citrate and/or isocitrate, while the α-ketoglutarate dehydrogenase mutant or any of the other mutants did not accumulate these compounds to a significant degree. While citrate or isocitrate might be responsible for the suppression of sporulation in the aconitase and isocitrate dehydrogenase mutants, they cannot be the cause of the very low sporulation frequency in the α-ketoglutarate dehydrogenase mutant nor in the other mutant strains.

2. Suppression of sporulation by glucose-6-phosphate. Dr. Prasad of this laboratory has constructed a triple mutant lacking the activities of glucose-6-phosphate dehydrogenase, phosphoglucoisomerase and phosphoglucomutase which can sporulate normally only in the absence of glucose. Its sporulation is extremely sensitive to suppression by very small amounts of glucose because the produced glucose-6-phosphate cannot be metabolized further. We have now shown by electron microscopic investigations that the mutant, grown under conditions of glucose-6-phosphate accumulation did not even start differentiation. Instead of forming asymmetric membrane septa, walls containing septa were formed randomly at many places of the cell, thus partitioning or sectioning off smaller or larger cell portions.

3. Abnormal differentiation of cacogenic mutants accumulating α-glycerol phosphate and curing by malate. Mutants of B. subtilis lacking glycerol phosphate dehydrogenase activity (NAD-independent) accumulated glycerol-P and could not sporulate. If the de novo pathway of glycerol production was intact, glycerol-P derived from the added carbohydrate, e.g., glucose. If the de novo pathway was blocked by another mutation, glycerol was required for growth. In both strains glycerol-P accumulated. Whereas the vegetative growth of the mutants was not significantly affected by the accumulation of glycerol-P, after the end of growth asymmetric septa appeared that were all filled with cell wall material. A single asymmetric septum not containing much cell wall material would normally suffice to allow the development of a spore in the bacterium. However, the presence of stiff cell wall in the mutant septum prevented all further invagination. The mutant cells subsequently made additional attempts to sporulate, but again produced walls containing septa. To exclude the

possibility that glycerol itself suppresses sporulation, additional mutations deficient in the conversion of glycerol to glycerol-P were introduced; glycerol was then unable to suppress sporulation.

The sporulation deficiency of the glycerol-P dehydrogenase mutant was completely cured by the addition of 40 mM malate to the growth medium. Under these conditions glycerol-P did not accumulate, because some metabolite derived from malate repressed the production of glycerol kinase and thus assured a low conversion of glycerol to glycerol-P. In the electron microscope the development of a single thin septum was observed which later invaginated and gave rise to the production of one spore per cell. The detailed reasons for the glycerol kinase repression are under investigation.

4. Sporulation deficiency of a mutant requiring glycerol for growth and methods restoring normal differentiation. A mutant that requires glycerol for growth because it apparently lacks glycerol-P dehydrogenase (NAD-dependent) was unable to sporulate under normal growth conditions. When the mutant was investigated under the electron microscope, a shrinking of the cell membrane from the cell wall was seen after growth had stopped. This observation suggested that the deficiency of glycerol-P may cause the formation of a thin membrane which bursts at some time during the development, releases part of the cytoplasm which is under higher pressure, and consequently reduces its extension, like a bursting balloon. In fact, the liberation of ATP at this time was observed. The problem with this interpretation was that the normal 168 strain of B. subtilis derives not only phospholipids but also a glycerol-teichoic acid from glycerol-P. In order to verify that the observed phenomena did not result from a deficiency in the teichoic acid production, another mutant was derived from a W23 strain in which teichoic acid contains ribitol instead of glycerol. Since the same observations were made in this strain, the observed deficiencies indeed seem to result from the lack of sufficient cell membrane production. Both types of mutants could sporulate normally and did not show the membrane shrinkage if small quantities of glycerol were frequently added during the developmental periods or if the growth medium contained both 10 mM glycerol and 40 mM malate, the malate again repressing the formation of glycerol kinase.

Significance to Biomedical Research and the Program of the Institute. The investigation of cacogenic mutants of microorganisms unable to differentiate normally is revealing a number of new principles which should be valid to a large degree also for hereditary diseases in humans. Particular cures of such deficiencies effective in bacteria may subsequently be attempted for similar mutations in humans. Especially intriguing is the phenomenon that malate can cure mutants accumulating glycerol-P and that it is useful for the curing of other mutants unable to make glycerol-P. The alteration of cell membranes must play an important role in a number of neurological diseases and in stroke. The methods developed in this laboratory to investigate such alterations and to prevent their occurrence should therefore be useful for the investigation of similar changes in higher organisms in which experimental manipulations are much more complex and frequently impossible.

Proposed Course of Project: Additional mutants will be analyzed to discover the mechanisms by which glycerol-P suppresses sporulation and by which malate represses glycerol kinase production. The process of differentiation and its deficiency in other cacogenic mutants will be investigated by electron microscopy, and the correlation of these results with biochemical investigations will be analyzed.

Honors and Awards: None

Publication:

Freese, E. B.: Unusual membranous structures in cytochrome *a*-deficient mutants of *Bacillus subtilis*. *J. Gen. Microbiol.* 75: 187-190, 1973.

Oh, Y. K., Freese, E. B., and Freese, E.: Abnormal septation and inhibition of sporulation by accumulation of L-α-glycerophosphate in *Bacillus subtilis* mutants. *J. Bacteriol.* 113: 1034-1045.

Serial No. NDS(I)-72 LMB/DB 1962
1. Lab. of Molecular Biology
2. Section on Developmental Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mechanism and Control of Membrane Transport

Previous Serial Number: SAME

Principal Investigator: Ernst Freese, Ph.D.

Other Investigator: Michael L. Hampton, Ph.D.

Cooperating Units: None

Man Years

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|---|---|
| Total: | 3.7 |
| Professional: | 3.3 |
| Other: | 0.4 |

Project Description

Objectives: Since it has become possible to isolate membrane vesicles from bacteria, one can study the transport of substrates through membranes independent of cytoplasmic components and compare it with that into whole cells. In this way it has been established that there is a close correlation between the transport of amino or keto acids and the electron transport system, but the presence or production of ATP is not required. The aim of this work is to establish how different energy sources energize transport of different substrates, by which compounds this transport can be inhibited, and how this knowledge can be employed to understand and improve the antimicrobial action of compounds important for Public Health. In particular, antimicrobial food additives have been studied with the attempt to understand their mode of action and to find alternatives to nitrite and sulfite, both of which are known to induce mutations in microorganisms and are suspected of carcinogenic potentialities in humans. Other lipophilic acids being studied are analgesics and antipyretics such as aspirin.

Methods Employed: Different compounds were screened for their inhibition of growth and oxygen consumption. In this way the inhibiting concentration range has been established. Membrane vesicles were isolated by protoplasting the cells with lysozyme and lysing them by rapid dilution. The further purification method was sufficiently refined to allow a simple and highly reproducible product. By growing the cells in the presence of glycerol, glycerophosphate dehydrogenase was induced, allowing glycerol phosphate to be used as energy source. This was important because in the presence of this energy source oxygen did not become rate limiting and many experimental tubes could be run at the same time.

Part of this work was performed on a contract with the Department of Microbiology, School of Medicine and Dentistry, Georgetown University, principal investigator, Dr. T. Sreevalsan The work reported here was done by the contract purchase of one man year.

*Major Findings*: 1. *Mechanism by which NADH energizes transport into membrane vesicles*. In membrane vesicles the amount of amino acid taken up per oxygen consumed was much highe with α-glycerophosphate than with NADH as energy source. This phenomenon has been variably explained by inefficient coupling o NADH oxidation to amino acid transport or by different electron transport pathways. The following observations show that these properties in fact result from the presence of open or inverted vesicles in the membrane preparation. Cells or protoplasts of *Bacillus subtilis*, preincubated to give very low endogenous consumption, oxidized 50 mM glycerol phosphate 13 times faster than 40 mM NADH, while cell breakage increased the rate of NADH oxidation to that of glycerol phosphate. Apparently glycerol phosphate entered cells more readily than NADH. While the oxidation of glycerol phosphate showed one apparent $K_m$ (6 mM), that of NADH showed two $K_m$'s (0.2 and 2.8 mM). With whole cells only the high $K_m$ was observed. Also the amino acid transport into membrane vesicles showed only the high $K_m$. Rotenone (0.4 mM) inhibited vesicular oxygen consumption much more than amino acid uptake. It is therefore concluded that NADH energizes amino aci uptake only from within the membrane vesicle. This explains the high $K_m$, because NADH penetrates the vesicular membrane only slowly.

2. *Inhibition of growth, amino acid transport, and oxygen consumption by lipophilic acids, including antimicrobial food additives*. Most antimicrobial food additives now used are lipophilic acids which inhibit growth of unwanted microorganisms. These compounds, as well as other lipophilic acids such as long chain fatty acids, all inhibit the growth of *Bacillus subtilis*, its consumption of oxygen, and its uptake of amino acids. In contrast, for Gram-negative bacteria only the small compounds of sizes up to hexanoic acid are inhibitory, whereas medium and lon chain fatty acids have no effect. The resistant to long chain

fatty acids can therefore be used to distinguish between Gram-positive and -negative organisms. The inhibitory strength of these compounds could be compared by plotting an inhibition index against the logarithm of the inhibitor concentration. It was found that the inhibition increased with the lipophilic character of the compound and with decreasing pH. Apparently, the inhibition was caused by the attachment of the free acid to the cell membrane.

3. Inhibition of transport into membrane vesicles. In isolated membrane vesicles, energized by glycerol phosphate or NADH, all of the above compounds inhibited the uptake of amino or keto acids; the inhibitory concentrations were the same as those for whole cells of B. subtilis. But most lipophilic acids did not inhibit the oxidation of NADH or glycerol phosphate, i.e., they did not inhibit the electron chain. No production of ATP was observed in the membrane vesicles. These results prove that the antimicrobial food additives and other lipophilic acids attach to the bacterial membrane and inhibit amino acid transport, apparently by uncoupling it from the electron transport chain, but without involving ATP. Some compounds (parabens) also inhibit partially the electron transport system itself, indicating that they affect cells by two different mechanisms.

4. Protection of Gram-negative bacteria against fatty acid inhibition by their lipopolysaccharide layer. Membrane vesicles of Gram-negative bacteria were as sensitive to long chain fatty acids as were those of B. subtilis. Intact Gram-negative bacteria are protected against long chain fatty acids that can usually be metabolized. But metabolism-deficient mutants are still resistant, indicating that the lipopolysaccharide layer may provide the protective screen. In fact, mutants of Escherichia coli or Salmonella typhimurium partially lacking the lipopolysaccharide layer (stripped from the KDO core out) were sensitive to inhibition by medium chain fatty acids such as decanoate with respect to both growth and amino acid transport. The differential inhibition by decanoate can therefore be used to distinguish between normal and mutant Gram-negative organisms. Furthermore, when the lipopolysaccharide layer was partially stripped off by EDTA treatment, the Gram-negative organisms became transiently sensitive also to long chain fatty acids but returned to their resistant state within 30 minutes of metabolism, during which the layer apparently could be repaired.

Significance to Biomedical Research and the Program of the Institute: Membrane transport is a universal feature of all cells, and any observations made on it in microorganisms should provide the essential features for transport mechanism and its inhibition in all organisms. Since antimicrobial food additives inhibit this transport in bacteria, it could be expected that they would also inhibit in mammalian cells, which has been verified in an accompanying report. The results can now be used to characterize new potential food additives, determine their relative strength, and analyze their potential harmful effects on nerve conduction and signal transmission, as well as fetal development. The sensitivity of Gram-negative bacteria to small lipophilic acids may be used to combat infections by these organisms.

Proposed Course of Project: For many other compounds which inhibit growth of microorganisms the mechanism of action is not known. The approach used here makes it possible to prove which compounds are specifically effective against the cell membrane and its transport properties. This will be investigated for antiseptics, for some antibiotics whose mode of action is unknown and also for analgesics and antipyretics. The mechanism by which the lipophilic acids inhibit membrane transport will be further investigated by the use of mutants and the isolation of membrane components. The results in microorganisms can be compared to transport studies and growth inhibition in human tissue cultures.

Honors and Awards: The principal investigator was reelected President of the Environmental Mutagen Society.

Publications:

Sheu, C. W. and Freese, E.: Effects of fatty acids on growth and envelope proteins of *Bacillus subtilis*. *J. Bacteriol*. 111: 516-524, 1972.

Sheu, C. W., Konings, W. N., and Freese, E.: Effects of acetate and other short-chain fatty acids on sugar and amino acid uptake of *Bacillus subtilis*. *J. Bacteriol*. 111: 525-530, 1972.

Sheu, C. W. and Ries, J. J.: A modified microtome for slicing polyacrylamide gels. *Anal. Biochem*. 49: 23-28, 1972.

Freese, E., Sheu, C. W., and Galliers, E.: How antimicrobial food additives work. *Nature* 241: 321-325, 1973.

Serial No. NDS(I)-72 LMB/DB 1963
1. Lab. of Molecular Biology
2. Section on Developmental Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Genetic Regulation in Human Diploid Tissue Culture

Previous Serial Number: SAME

Principal Investigators: Jeffrey L. Simmons, M.D. and
Ernst Freese, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

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|---|---|
| Total: | 1.6 |
| Professional: | 1.6 |
| Other: | 0 |

Project Description:

Objectives: Recent developments in mammalian cell techniques have made it possible to develop methods whereby the genetic and developmental control of cell alterations in mammalian cells can be studied. Attempts in this laboratory to investigate a frequent hereditary disease, homocystinurea, indicated a possible abnormality of amino acid substrate transport. In addition, studies on the effect of lipophilic acids, including food additives in bacteria, had indicated that these compounds inhibit the amino acid transport. Therefore, a systematic investigation of the transport properties of normal human diploid fibroblasts was launched in order to correlate this information with abnormal cells and to study its inhibition by lipophilic acids. In addition, the growth inhibitory effect and other properties of lipophilic acids on different human cells were examined.

Methods Employed: Amino acid transport measurements are usually performed by artificially suspending the cells and exposing them to radioactive substrates for a defined period. A number of difficulties arise in this approach: 1) the cells are removed from their normal physical environment; 2) they must usually be exposed to some digestive compound (e.g., trypsin) to achieve adequate separation; 3) rapid measurements to determine initial rate kinetics are often not feasible because of physical limitations. We have used a cover slip monolayer method, which is remarkably flexible and reproducible. In addition, we have labeled the cells for long times with a tritiated compound in order to monitor the number of cells; the incorporation of a $^{14}$C compound is then measured by evaluating each radioactive label separately in a scintillation counter. Cell columes could be determined by comparing inulin and urea spaces.

For the examination of the effect of lipophilic acids on human cell growth and shape, a contract was used with the Department of Microbiology, School of Medicine and Dentistry, Georgetown University, principal investigator Dr. T. Sreevalsan. The work done by this contract involved about two man years. The cells were investigated under an inverted phase contrast microscope, their number was counted by a hemocytometer, and dead cells were identified by staining with Trypan blue. The various lipophilic acids were dissolved either in water, for the small molecular weight compounds, or in dimethylsulfoxide.

Major Findings: 1. Inhibition of amino acid transport. Similar to the "A" and "L" transport systems originally described by Christensen, we have found specific transport systems in human fibroblasts. Whereas the transport of arginine was sodium-independent, that of alanine required sodium and was capable of maintaining normal transport in spite of the presence of metabolic inhibitors if an adequate sodium gradient was present. This transport of alanine continued even after the ATP levels had fallen one-thousandfold. Sodium could not be replaced with lithium, potassium, ammonium, Tris, or choline, and the transport ceased when sodium was removed from the medium. Only when the sodium level was suboptimal did transport become inhibitable by cyanide, DNP, or ouabain, indicating that at this level of external sodium a gradient of sodium necessary for transport can be maintained only in the presence of an active Na-K-ATPase. Initial experiments with lipophilic acids have also shown such inhibition.

2. Effect of lipophilic acids on growth of HeLa cells. The inhibition of growth and killing of HeLa cells by different lipophilic acids have shown that their effect increased with the concentration of the compound and with their lipophilic tendency and that all of these compounds are at least as effective as in Bacillus subtilis. At very high concentrations all compounds were lethal to the cells. These results have clearly establishe that antimicrobial food additives are as effective against human

cells as against bacteria and indicate that too extensive use of such compounds may be harmful to the function of some human cells. In fact, intestinal cells probably are not seriously exposed to the lipophilic acids ingested with food, only because these compounds are rapidly removed into the blood stream by an ion concentration mechanism which depends on the low pH in the gut and the neutral pH in the tissue.

3. Morphological alterations induced by lipophilic acids in epithelial-like cells. Apart from their growth inhibitory property, some of the smaller lipophilic acids produce pronounced morphological alterations in HeLa cells and other epithelial-like cells. For example, when HeLa cells were incubated in the presence of 5 mM butyrate, filamentous protrusions appeared after 7-8 hours and had developed into long spikes by 14 hours. Propionate, hexanoate, methyl- or propyl-parabens produced the same morphological changes as butyrate. However, longer chain fatty acids or 2,4-dinitrophenol did not have this effect. Under conditions under which the cells were morphologically altered, viability did not significantly change for up to 96 hours, and the cell shape returned to normal as soon as the inhibitory compound was removed. Addition of cAMP or But-cAMP did not cause such alterations, nor did they have the frequency of butyrate-induced morphological changes; But-cAMP has previously been shown to affect fibroblasts but not epithelial-like cells. The effects of lipophilic acids and cAMP therefore seem to involve mutually exclusive cell types.

4. Effect of butyrate on growth of animal viruses. The capacity of HeLa cells to support the growth of Sindbis or vesicular stomatitis virus, both enveloped riboviruses, is greatly reduced by treatment of HeLa cells with butyrate. Yet the growth of other riboviruses such as polio, is unaffected. Little or no virus-specific RNA is synthesized in butyrate-treated HeLa cells subsequent to infection with Sindbis virus. Apparently, absorption, penetration, uncoating, or maturation of Sindbis virus are unaffected, while butyrate inhibits the ability of the viral RNA to replicate.

Significance to Biomedical Research and the Program of the Institute: The understanding of membrane properties is important for a host of human diseases. In a number of hereditary diseases, including amino acidurias, amino acid transport may be impaired. Cancer cells have altered membranes and may be affected in their transport properties. Teratogenic effects may be caused by a number of compounds, including lipophilic acids and drugs and possibly even compounds obtained in too large quantities in our food. The effect of antimicrobial food additives certainly shows that human cells are as sensitive to lipophilic acids as bacteria and they indicate the need to monitor food additives not only in bacteria but also in mammalian tissue cultures, so that only compounds are used that are either relatively ineffective on human cells or can be sufficiently rapidly metabolized.

Proposed Course of Project: The amino acid transport of human cells will be examined to determine the energy requirements of cells and to elucidate the importance of the sodium pump in transport. The transport will be measured in both normal and virus-transformed cells and its inhibition will be determined in the presence of lipophilic acids. The shape changes caused by some of the lipophilic acids were unexpected and require exploration. They may be related to transport properties of the cells or be caused by the effect of these low molecular weight compounds on some biosynthetic reactions. The fact that lipophilic acids inhibit some viruses and not others is remarkable and could potentially lead to the cure of some virus diseases. This phenomenon will be investigated for a number of additional mammalian viruses. The effect of lipophilic acids, such as antiseptics, analgesics, and antipyretics will also be investigated, because their biochemical nature makes it likely that they have similar interactions with mammalian cell membranes.

Honors and Awards: None

Publications:

Ginsburg, E., Salomon, D., Sreevalsan, T., and Freese, E.: Growth inhibition and morphological changes caused by lipophilic acids in mammalian cells. Proc. Natl. Acad. Sci. USA, in press.

Serial No. NDS(I)-65 LMB/RN 1208
1. Lab. of Molecular Biology
2. Section on Regulation of Nucleic Acid Synthesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ribonucleic Acid and the Regulation of Cellular Metabolism

Previous Serial Number: SAME

Principal Investigator: Robert A. Lazzarini, Ph.D.

Other Investigators: Leonard E. Maroun, Ph.D., Yoshikatsu Murooka, Ph.D. (Visiting Fellow), Gudrun Stamminger, Ph.D. (Guest Worker)

Cooperating Units: None

Man Years

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|---|---|
| Total: | 1.5 |
| Professional: | 1.25 |
| Other: | .25 |

Project Description:

Objectives: The nucleic acid content of cells is subject to control by several regulatory mechanisms. The synthesis of certain species of messenger RNA is elicited by the presence or absence of specific metabolites in the cell milieu. The accumulation of ribosomal RNA, however, does not respond to the availability of specific nutrients but responds, rather, to the fitness of the medium for growth and the growth rate of the cell. Transfer RNA, on the other hand, responds neither to specific nutrients nor to the growth rate but is constant under most conditions of growth. The details of the mechanisms underlying these observations are at present obscure and are the subject of this project. We have concentrated on three aspects of these control mechanisms: 1) Control of ribosomal RNA synthesis in growing and nongrowing microbial cells. 2) The cell-free transcription of ribosomal RNA. 3) Control of ribosomal RNA during early embryogenesis.

Major Findings: A wealth of experimental evidence has been amassed suggesting that the intracellular concentration of the guanosine tetraphosphate, ppGpp, somehow modulates ribosomal RNA synthesis. Attempts to test this notion have been difficult. RNA synthesized by purified RNA polymerase and DNA typically contains only 2-10% rRNA sequences, as compared with 50% observed in growing cells. This low relative rate of rRNA synthesis is reminiscent of that observed in the fully repressed in vivo system--the amino acid starved stringent E. coli--and may respond to a basal or unstimulated rRNA synthesis in vivo. A critical in vitro test of this proposal requires an assemblage of a complete complement of enzymic and structural elements necessary for a high relative rate of rRNA synthesis under conditions where the concentration of the putative effector molecule, ppGpp, can be manipulated. In order to test this hypothesis in whole cells, mutants deficient in their ability to regulate ppGpp concentrations or rRNA synthesis can be employed. These might include temperature sensitive mutants which at the restrictive temperature are enabled to control rRNA synthesis or degradation of ppGpp. We have employed all three approaches in an effort to test the hypothesis.

1. Cell-free synthesis of rRNA. We have continued the work on rRNA synthesis with permeabilized E. coli cells and with a membrane-DNA-RNA polymerase complex isolated from these cells. Both of these systems synthesize rRNA at the expense of added nucleoside triphosphates at a high relative rate. Neither of these systems, however, shows a selective sensitivity to the putative effector molecule, ppGpp. Although this nucleotide inhibits total RNA synthesis by 50%, a specific inhibition of rRNA synthesis was not observed. We conclude, therefore, that ppGpp does not directly and by itself inhibit the enzymatic elements necessary for derepressed rRNA synthesis. If ppGpp regulates rRNA synthesis in vivo it seems likely that it does so through the agency of some factor that is missing or inactivated in the in vitro systems examined.

2. Thermo-sensitive mutants defective in RNA synthesis. We have isolated temperature sensitive mutants of a relaxed E. coli strain which upon transfer to the restrictive temperature first preferentially restrict their synthesis of rRNA and after 30 minutes their synthesis of total RNA. This discoordinate regulation of RNA synthesis yields an enrichment in the protein to RNA ratio of the cell. The restriction on rRNA synthesis is accompanied by a modest accumulation of ppGpp. The intracellular levels of the four nucleoside triphosphates are not altered by the temperature shift, suggesting that the curtailment of RNA synthesis is not a result of a substrate deficiency. However, after permeabilization the mutant and parent strain synthesized mRNA and rRNA at the expense of exogenous nucleoside triphosphates at the same rate, irrespective of the culture temperature prior to permeabilization. These results strongly suggest

that an easily diffusable molecule restricts rRNA synthesis at the elevated temperature and upon permeabilization of the cell diffuses out allowing RNA synthesis to resume.

3. Mutants with altered metabolism of guanosine tetraphosphate. A most important characteristic of microbial cells is their ability to rapidly adjust, either upwards or downwards, their intracellular ppGpp levels. We have obtained a mutant which retains its ability to rapidly accumulate ppGpp but is impaired in its ability to reduce a once elevated level of this nucleotide. The observed rate of degradation of ppGpp at this mutant are 1/20th to 1/40th the rate observed in wild type strains. This mutant has a second characteristic which sets it apart from the wild type strain. High levels of the antibiotic oxytetracycline completely inhibit the metabolism of ppGpp in the mutant. We suspect that both phenotypes are derived from the same genetic lesion. The finding that an inhibitor of protein synthesis, oxytetracycline, effectively blocks the degradation of ppGpp is a provocative one. It suggests that ribosomes or the protein synthesis apparatus of the cell might not only be responsible for the synthesis of ppGpp but also its further metabolism. The notion that ppGpp regulates rRNA synthesis is considerably strengthened by experiments carried out with this mutant. In the first of these the mutant was starved for a required amino acid which elicited a high intracellular concentration of ppGpp and then was returned to complete medium. Protein synthesis began almost immediately and the turbidity of the culture increased within 5 minutes of the return to complete media. However, it was not until the ppGpp levels fell too close to the basal amount (approximately 25 minutes after return to complete medium) that net RNA accumulation commenced. Consequently, amino acids efficiency and active protein synthesis are not by themselves sufficient conditions for net RNA synthesis. In a similar experiment the antibiotic oxytetracycline was added to maintain the intracellular ppGpp level even in the presence of complete medium. Under these conditions rRNA synthesis does not resume even though complete media is present.

Significance to biomedical research and the program of the Institute: Controls of nucleic acid synthesis in bacterial systems represent examples of regulatory mechanisms which are more amenable to experimental investigation than those of the more slowly-growing mammalian systems. It is anticipated that information obtained from these simpler systems will help form a basis upon which an investigation of the more complex regulatory mechanisms of higher organisms can be built and eventually an understanding and control of disease in these systems can be obtained.

Proposed Course of Project: To further investigate the cell-free synthesis of ribosomal RNA using both the mutant and wild type cells as a source of enzyme. To identify the thermo-labile element in the mutant strain. To investigate the enzymic components necessary for the further metabolism of ppGpp in mutant and wild type cells.

Honors and Awards: None

Publications:

Murooka, Y. and Lazzarini, R. A.: Stimulation of RNA synthesis by two protein factors in extracts of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 69: 2336-2340, 1972.

Lazzarini, R. A. and Johnson, L. D.: On the regulation of RNA synthesis in cold-shocked *E. coli* cells. *Nature New Biology*, in press.

Murooka, Y. and Lazzarini, R. A.: *In vitro* synthesis of ribosomal RNA by a DNA-protein complex isolated from *Escherichia coli*. *J. Biol. Chem.*, in press.

2. Virus RNA synthesis. VSV virions contain an RNA-dependent RNA polymerase which in detergent-treated virions will transcribe the virus RNA. We have examined the product of this reaction and have discovered that at least 1/3 of the RNA synthesized contains polyadenylate [poly(A)] tracts. We have succeeded in separating these poly(A)-containing RNA's by taking advantage of the fact that they immediately hybridize with and bind to oligo-dT-cellulose. We are currently investigating whether the virion RNA polymerase synthesized the poly(A) tracts or whether a second enzyme is responsible for the synthesis. If the RNA polymerase itself synthesizes the tracts, then it seems likely that they are coded for in the virus genome.

We have also found that viral RNA produced during the infection of a host cell contains poly(A) tracts. This RNA completely hybridizes with RNA isolated from the virion indicating that it, like the RNA produced *in vitro*, is complementary to the virion RNA. Consequently, the RNA synthesized *in vitro* by detergent-treated virions is not aberrant or unnatural but appears to be an accurate reflection of the early RNA synthesis in the infected cell. Since this particular kind of synthesis, RNA-dependent RNA synthesis, is not carried out in the uninfected host cell, this viral process probably represents the most accessible and specific target for therapeutic agents. Our results suggest that RNA synthesis by detergent-treated VSV virions could be employed as a simple screen for chemotherapeutic agents which interfere with viral RNA synthesis.

3. Significance to biomedical research and the program of the Institute. Viral diseases of the central nervous system (CNS) usually occur as a complication rather than a normal consequence of infection. Nevertheless many members of the myxo-, paramyxo-, rhabdo-virus family, either exceptionally or as a normal consequence, infect the CNS causing encephalitis or meningitis. For example, the myxo-virus, fowl plague virus, can cause a diffuse encephalitis. Examples within the paramyxo virus family are more numerous and include the viruses causing mumps, measles, canine distemper, and Newcastle's disease. Rabies virus is an outstanding example in the rhabdo-family. Despite their importance to medical neurology very little is known about the regulation and mode of replication of these viruses in the host organism. The study of viral infections in cultured cells will reveal the molecular biological characteristics of the replication in a manner uncomplicated by the host response to the infection. It is anticipated that the study will delineate characteristics that can be exploited in containing and limiting viral infection to non-neural tissues or in the treatment or prevention of the viral infection.

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Proposed course of Project. 1) Investigate the nature of the template for viral mRNA synthesis to determine if it is single or double-stranded RNA. 2) Investigate the role of the protein kinase in the infectious process. 3) Determine the number of RNA polymerase molecules per virion, and the rate at which they elongate RNA chains.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1972 through June 30, 1973
Central Nervous System Studies Laboratory
National Institute of Neurological Diseases and Stroke
National Institutes of Health

Laboratory Chief: D. Carleton Gajdusek, M.D.

This laboratory has two closely interrelated projects which diverge at their technical extremes into very different disciplines. However, they have fertilized each other since their origin, and the second has grown from the first.

The same investigators are involved in both projects, and their creative stimulus, dedication and enthusiasm, in part, at least, are the product of the intellectual stimulation that the diverse approaches phrased by the two projects provide. The projects are:

Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures.

Chronic Central Nervous System Disease Studies: Study of Slow, Latent and Temperate Virus Infections.

## Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primive Cultures

Principal Investigators: D. Carleton Gajdusek and C.J. Gibbs, Jr.

This section has continued to focus its attention on long term studies of the human biology of the many vanishing primitive societies. Our laboratory research and that of our numerous collaborating investigators, is directed to problems of the neurological development and learning patterns in children in diverse cultural experiments in the human condition found in isolated primitive populations. Laboratory studies on human biology, genetics and associated molecular biology, on immunology, virology and biochemistry have all been directed at solving problems which have been carefully chosen from small isolated bands still living in the primitive situation in which these problems may be more appropriately studied than in larger civilized societies. Nutritional studies, studies on reproduction and fertility, studies of neuroendocrine influences on age of sexual maturation and aging, on studies of the selective advantage and establishment of genetic polymorphisms are all underway. The unusual and odd alternatives of employing the central nervous system in its higher cerebral function of patterns of learning, language, cognitive styles, computation (number sense and calculation without a number system), and psychosexual culturally-modified behavior, are providing data on alternative forms of possible neurological functioning for man, of which we would remain unaware, and for moral, ethical, and political reasons be unable to produce or investigate in the clinic or laboratory, once the natural cultural experiments in primitive human population isolates have finally been amalgamated into the modern civilized cultural veneer which is now imposed upon almost all members of the community of man.

Growth and development studies in primitive cultures have yielded evidence for delayed puberty and slow growth rates in certain peoples, particularly the short-statured populations in the Highlands of New Guinea. Preliminary evidence suggests high levels of pituitary growth hormone in such populations in both pituitary glands obtained at autopsy, and in serum specimens. The growth rate seems to be proportional and age of puberty inversely proportional to mean adult stature. Congenital mental defect, and a wide range of neurological problems associated with the most severe foci of endemic cretinism in the world, in the highlands of West New Guinea, are under further investigation. These are contrasted with enclaves of familial goitrous cretinism in coastal populations on samll islands. The frequent, but irregular association of familial deaf mutism with goitrous cretinism is under study.

The discovery of new genetic factors, including haptoglobins, hemoglobins, and red cell enzyme pleomorphisms, with the elucidation of their biochemical structure and their later use as markers in human population genetic studies has been a byproduct of these investigations. The discovery of immunologically virgin populations without exposure to respiratory and enteroviruses which are ubiquitous in the civilized world has permitted fundamental investigation of the immune response in man, investigations possible nowhere else on the persistence of the immune response after natural measles infection and live attenuated measles virus immunization in the absence of circulating virus, yielding data important to the diagnosis of understanding of delayed slow measles encephalitis (subacute sclerosing panencephalitis, or SSPE), and establishing the serological identity of the agent of the 1918 influenza pandemic to aid in the genetic-historical elucidation of the serial mutation of influenza virus. By virtue of limited travel during their entire lifetime and intensive exposure to their natural ecology, members of primitive groups serve as unequaled sentinel populations for revealing the focal microbial agents that infect man in their environment. Thus, for infections ranging from Chagas disease and toxoplasmosis to arbovirus infections, primitive groups offer unusually fruitful samples for investigation; on toxoplasmosis, filariasis, yaws and malaria, as well as arbovirus encephalitides, we have studies in progress.

With the current interest in papovaviruses (SV40, J-C, BK) latent in man as the cause of progressive multifocal leucoencephalopathy, the presence and the pattern of acquisition of antibodies to these in isolated primitive groups, with no contact either with primates or vaccines, help settle the problem of the origin of these infections in man. Similar antibody acquisition patterns for cytomegalovirus, Epstein-Barr virus, and herpes viruses types 1 and 2, and other simian, including foamy viruses are also being studied in these isolates to add to our knowledge of their biological role.

Our efforts to document the development and neurological patterning in the disappearing primitive cultures has resulted in one of the largest archives of such documentation in the world. Collections and preservation of existing cinema data from New Guinea, Oceania, African aborigines, Australian aborigines, and American Indian groups, as they live as hunter-gatherers or primitive hoe-and-digging-stick agriculturists, provides the only such documentation of the life and behavior of man as he lived and evolved for most of his evolutionary history, about a million years. Our various laboratory investigations have evolved around the theme of elucidating medical problems in the cultures of primitive man or man living in small, isolated communities by carefully selecting such problems of immunological, virological, biochemical, genetic, reproductive biological, endocrinological and nutritional interest, all pertinent to the long-term studies of growth, behavior, emotional response to stress, and human biology in these groups.

Potent tools of mathematical population genetics have been used with our massive genetic pleomorphism data on primitive groups for the analysis of the multi-locus genetic effects in determining expression of neurological diseases such as kuru, and a similar approach is under way for the transmissible virus dementias. Genetic distance techniques, including genetic tree construction and principal components analyses, are used.

Our studies in the British Solomon Islands and the New Hebrides, and those in the Caroline Islands of Micronesia, were greatly enhanced this year by the opportunity to use the Research Vessel Alpha Helix of the Scripps Institution of Oceanography and the support of the National Science Foundation for our medical and population genetic studies in these islands, with a large team of investigators from our laboratories and those of our close collaborators.

## Chronic Central Nervous System Disease Studies: Study of Slow, Latent and Temperate Virus Infections

Principal Investigators: D. Carleton Gajdusek and C.J. Gibbs, Jr.

The aim of this laboratory has been, since its inception, to elucidate the causes and pathogenesis of subacute and chronic degenerative disorders of the nervous system. Multiple sclerosis, amyotrophic lateral sclerosis, parkinson's disease, Alzheimer's disease and other presenile dementias have been a major focus. However, the significant advances have occurred on the periphery of the spectrum of these degenerative CNS disease. Thus, kuru, the first chronic disease of man demonstrated to be of slow virus etiology, led us to the demonstration that presenile dementias of the Creutzfeldt-Jakob type were caused by a similar virus. The further pursuit of this lead has made possible the definition of the subacute spongiform virus encephalopathies as a group of related diseases of man (kuru and C-J disease) and animals (scrapie of sheep and transmissible mink encephalopathy). During the past two years further study of patients with progressive dementia has provided the surprising demonstration that even the heredofamilial form of C-J disease is transmissible and of virus etiology. Although most cases are sporadic, there are families in which the disease has occurred in siblings, parents, and close maternal and/or paternal relatives over several generations. From two such families the disease has now been experimentally transmitted to primates, demonstrating, for the first time in man, that disease with apparently genetic determination may be caused by a slow virus. This has added impetus to our already extensive studies of Huntington's chorea, Alzheimer's and Pick's diseases, parkinsonism-dementia, and even senile dementia. These investigations have so broadened the picture of C-J disease that we now must refer to transmissible virus dementias. This is necessary since the diseases from patients with subacute or chronic dementia, whose clinical and pathological diagnoses have been Alzheimer's disease and papulosis atrophicans maligna of Degos, have been transmitted to primates as subacute spongiform encephalopathies. Thus, the trail from kuru to C-J disease now embraces studies of presenile and senile dementias of all sorts, including "dementia praecox", the organic brain disease associated with late, uncontrolled schizophrenia.

The demonstration that non-inflammatory chronic degenerative, even heredofamilial CNS disorders, could be of virus etiology greatly stimulates the search for possible virus etiology in the demyelinating and sclerosing CNS diseases and chronic or actual encephalitides. The seroepidemiological approach to multiple sclerosis, with antibody determinations in the spinal fluid and serum, has led to our conviction that at least some of the patients are suffering from delayed and slow defective virus infections, probably with the measles virus, as Adams and others have earlier suggested. Intensive studies with whole virus and virus subunit antigens of the serial spinal fluid and sera, and gammaglobulin extracted from autopsy brain specimens for antibody activity is under way. Search for such antigens in brain tissue is in progress.

The two disorders involving white matter which have been demonstrated to be of slow virus etiology are 1) delayed and slow measles encephalitis (SSPE), and 2) progressive multifocal leucoencephalopathy (PML), from which two papovaviruses, SV 40 and J.C., have now been isolated. The large series of brain biopsy and autopsy specimens, sera and spinal fluid we had assembled on these diseases have materially contributed to their elucidation. It now appears that even certain forms of chronic progressive epilepsy are caused by slowly progressive chronic focal encephalitis. We have in the laboratory an experimental model in rhesus monkeys for this disease, using a strain of tick encephalitis virus (RSSE) which causes, after recovery from the acute encephalitis, slowly progressive movement disorders and focal myoclonic epilepsy. Thus, the range of chronic degenerative CNS diseases which must be further studied from a virological and immunological approach have been extended.

In an attempt to unmask viruses in these diseases brain cells have been grown _in vitro_ by both primary explant and trypsinized cell layer techniques from specimens obtained at biopsy and early autopsy. Co-cultivation and cell fusion and BUDR or IUDR induction are major techniques employed. The presence of a virus in these cultures is sought by electron microscopic scanning, staining for inclusion bodies, search for CPE, hemadsorption, fluorescent antibody, interferon production, virus interference and animal inoculations. The same techniques are applied to surgically sterile tissue obtained from experimental animals affected with kuru, C-J disease and other slow virus infections. As a by-product to the work many masked and latent viruses from chimpanzee and monkey hosts have been isolated. These have included reoviruses and many new adenoviruses, several new Simian herpesviruses (including cytomegaloviruses and Epstein Barr virus), and a wide series of new foamy viruses. These latter foamy viruses are of particular interest, in that they are oncornaviruses characterized by the production of a reverse transcriptase, yet they have not caused cell transformation _in vitro_, or tumors in monkeys. It has been shown that they are present in the brain and other organs of most chimpanzees and monkeys.

The long maintenance of brain cells, presumably astroglial cells, dedifferentiated on passage to resemble fibroblasts, regularly result in cell lines which propagate indefinitely and show many properties that suggest transformation. More blatant transformation, with full loss of contact inhibition, has occurred in three episodes with human brain cells: in the first instance, from a C-J disease patient's brain tissue, with regular presence of a Mason-Phizer monkey virus-like MP-MV oncornavirus in the transformed human brain cells (Hooks et al., 1971, 1973). The other two transformations have occurred in two brain cell lines from C-J patients which transformed with full loss of contact inhibition, but without the presence of recognizable virions in the transformed cells (Nancy Rogers and V. ter Meulen, unpublished data). These transformations have given further support to the speculation that the slow virus-cell interaction in the degenerative CNS diseases may be closely related to the slow virus-cell interaction in cell transformation and carcinogenesis. The use of the term "temperate" in naming our laboratory in 1962 seems to have been prophetic.

A group of atypical viruses, unlike any others known to microbiologists, appear to cause the subacute spongiform virus encephalopathies. The other CNS degenerations caused by slow viruses are caused by rumbling nonproductive, even defective infection with the more conventional measles, papovavirus(SV40 and J.C.), rubella virus, cytomegalovirus, herpes simplex virus, adenovirus type 32, and RSSE. The atypical viruses (kuru, C-J disease, scrapie, TME) have unusual resistance to ultraviolet radiation and ionizing radiation, to heat, to proteases and nucleases, and to formaldehyde. They are not associated with a recognizable virion on electron microscopic study of infected cells _in vivo_ or _in vitro_ or concentrated virus preparations (zonal banding). This has led to the speculation that they represent infectious agents, lacking a nucleic acid, perhaps even a self-replicating membrane fragment. A major effort of this laboratory has been, and is now being directed toward the molecular biological elucidation of the nature and structure of this group of atypical viruses. They have been partially purified (fluorocarbon protein precipitation) and successfully concentrated in zonal density banding in the ultracentrifuge and other physical and chemical properties and EM structure is under study. The solution of their structure is of immense importance to general microbiology, since they represent a new type of transmissible agent unlike any of the other agents with which we work in virology. We currently believe that they are composed of very small units of infectious nucleic acid, perhaps under 100,000 daltons, attached by strong binding to fragments of normal plasma membrane usually without geometrically recognizable packaging. The tight binding to the plasma membrane must confer the unique high U/V resistance and nuclease resistance to the nucleic acid moiety. Experiments are under way to refute or substantiate this view of their structure, which has been suggested by the preliminary studies.

Serial No. NDS (IR)-62 OD 969
1. Intramural Research
2. Central Nervous System Studies Laboratory
3. Bethesda, Maryland

PHS - NIH
Project Report
July 1, 1972 through June 30, 1973

Project Title: Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infections

Principal Investigators: D. Carleton Gajdusek, M.D.
Clarence J. Gibbs, Jr., Ph.D.

Sub-Project I: Attempts to isolate, identify and characterize transmissible agents from humans and animals with subacute degenerative diseases of the central nervous system: transmissible heredofamilial diseases, pre-senile and senile dementias of the sporadic and familial types and primary sclerosing and demyelinating diseases.

Sub-Project II: Characterization and pathogenesis of kuru virus.

Sub-Project III: Characterization and pathogenesis of Creutzfeldt-Jakob disease (transmissible dementia) virus.

Sub-Project IV: Studies on the characterization and nature of the viruses of scrapie, transmissible mink encephalopathy and visna diseases.

Sub-Project V: *In vivo* and *in vitro* host range of the subacute spongiform virus encephalopathies.

Sub-Project VI: Studies on Australian antigen in chimpanzees.

Sub-Project VII: Fluorescent antibody studies on the intracellular localization and identification of viral antigens *in vivo* and *in vitro* in tissues from patients with subacute diseases of the CNS.

Sub-Project VIII: Tissue and cell culture *in vitro* studies of virus induced slow infections of man and animals.

Sub-Project IX: The simian foamy viruses.

Sub-Project X: Studies on transformed human brain tissue *in vitro* and characterization of associated virus.

Sub-Project XI: Isolation and characterization of cytomegalovirus from the urine of chimpanzees.

Sub-Project XII: Characterization and identification of new herpes viruses from explant cultures of tissues from sub-human primates.

Sub-Project XIII: Studies on persistent asymptomatic cytomegalovirus infections of healthy rhesus monkeys.

Sub-Project XIV: Focal movement disorders in rhesus monkeys following experimental infection with a strain of tick-borne encephalitis virus.

Sub-Project XV: Studies on the polymorphonuclear depressing factor in multiple sclerosis and other slow infections of the nervous system.

Sub-Project XVI: Development of serological and immunological test system for use in the study of slow infections of the CNS.

Sub-Project XVII: Immune responsiveness of multiple sclerosis patients to established viral antigens by detection of specific antibodies in serum and cerebrospinal fluids collected serially during remissions and exacerbations.

Sub-Project XVIII: Animal management and intercurrent diseases in sub-human primates on long-term studies of slow infections.

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Administrative: Marion Poms, Juliette Harvey, Linda Poole, Lois Ludeman

Man Year:

| | |
|---|---|
| Total: | 38 |
| Professional: | 26 |
| Others: | 12 |

Publications: Yes

Project Description: The Central Nervous System Studies Laboratory, under direction of the co-principal investigators, has as one of its two projects an integrated team study on Slow, Latent, Temperate, Masked, Persistent and Chronic Diseases of Man and Animals, with particular emphasis on those affecting the nervous system. The major diseases under study are: kuru, Creutzfeldt-Jakob disease, and other presenile and senile degenerative processes affecting man, Pick's, Alper's, Alzheimer's diseases, Huntington's chorea, epilepsia partialis continua, multiple sclerosis, amyotrophic lateral sclerosis, Parkinsonism-dementia, progressive supranuclear palsy, subacute sclerosing panencephalitis (SSPE), progressive multifocal leucoencephalopathy, Schilder's disease, chronic encephalitis, transmissible papulosis atrophicans of Degos, dementia praecox associated with late uncontrolled schizophrenia. As may be noted in the preamble to this report there are 18 subprojects that make up the scope of the work.

Objectives: The major objectives established in 1961 have not substantially changed. They remain as follows: to elicit the etiology of subacute progressive degenerative diseases of the nervous system of man and animals; to study the pathogenesis of these diseases, their natural method of spread, and their seroepidemiology; to determine the role of the genetic mechanism of the host as it relates to resistance and susceptibility to disease; to determine the natural and experimental host range; to determine the nature of the viruses isolated from naturally and experimentally infected hosts through studies on the physical, chemical, biological, immunological, histochemical, histopathological and genetic mapping properties of the agents causing these diseases; to study the mechanisms of helper and hinderer viruses that may be involved in the pathogenesis of these diseases; to elicit immunopathogenic mechanisms involved, including neoantigens at the cell surface.

Methods Employed: The methods employed in these studies, with necessary alterations to meet the needs of specific problems encountered, remain essentially the same as described in previous reports. The utilization of techniques developed in this program for the isolation and characterization of "atypical" viruses, ubiquitous viruses and other infectious agents from human tissues and animal tissues, particularly from the nervous system of the affected host have been described. Such techniques include inoculation and long-term holding of higher sub-human primates and monkeys, domestic animals and avian hosts and a wide variety of smaller mammalian and avian species in the laboratory; development, establishment and utilization of in vitro tissue and cell culture systems from naturally affected humans and animals in which diseases have been induced as well as the utilization of primary and continuous cell lines from a wide variety of human, animal and avian tissues for rescuing viruses; utilization of biophysical, biochemical, histological, histochemical, immunological and immunochemical techniques in an effort to characterize isolates or provide markers for the in vivo detection of latent/persistent defective viruses. Contractural phases of this work are being conducted at:

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| Gulf South Research Institute, New Iberia, Louisiana | 280,000.00 |
| National Center for Primate Biology, University of California, Davis | 40,000.00 |
| Public Health Research Institute of New York, Otisville | 119,000.00 |
| Max-Planck Institute for Neuropathology, Frankfurt, Germany | 13,000.00 |

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| Department of Pathology, University of California San Diego | 4,450.00 |
| Department of Microbiology, University of Western Australia, Perth | 2,500.00 |
| Department of Virology, Hopital Salpetriere, Paris | 2,500.00 |

This project is part of the Central Nervous System Studies Laboratory (see Project Report Serial No. NDS (IR)-1282 (I-XI).

*Major Findings*: These studies have established infection as the etiology for a number of subacute progressive degenerative diseases of the nervous system of man and animals. The data collected have provided the information necessary to classify slow infections of the nervous system into two broad categories based on the nature of the disease and the physical, biological and immunological properties of the viruses which cause them: (1) "atypical" diseases caused by highly unconventional viruses which form a new group of infectious agents and which we have designated the subacute spongiform virus encephalopathies - kuru and transmissible dementia of the Creutzfeldt-Jakob type of man and scrapie and transmissible mink encephalopathy of animals; and, (2) fatal CNS diseases with a protracted course associated with elevated proteins and CSF pleocytosis, histological evidence of inflammatory response and an immune response detectable in serum and CSF with titer ratios between CSF and serum frequently in excess of the ratios for other virus antibodies: defective measles in SSPE, papovaviruses, including J.D., B.K., and $SV_{40}$ in PML, rubella virus in congenital CNS infections, chronic encephalitis from herpes simplex and adenovirus type 32, and chronic distemper in dogs, focal movement disorders in primates with RSSE virus and the probability of measles and related viruses in some forms of multiple sclerosis.

The basic study of kuru has provided for the isolation and characterization of over two hundred virus strains from *in vitro* explanted tissues, primarily animal in origin: the development of a transformed human brain cell line associated with an RNA virus having a reverse transcriptase which is antigenically related to the Mason-Pfizer monkey mammary tumor virus; isolation of additional new simian foamy viruses and a clearer definition of the properties of these viruses as members of the group oncornaviruses. These studies have further led investigators in other laboratories to the successful isolation of the viruses of SSPE and PML.

*Kuru*: a heredofamilial disease of the Fore people of the eastern highlands of Papua New Guinea. In man the incubation period is estimated to be from 5 to more than 20 years following self inoculation during the practice of ritualistic cannabalism. The disease is transmissible to chimpanzees (incubation period 10-59 months), 5 species of new world monkeys (spider, 10-51 months; squirrel, 9-44 months; capuchin, 10-45 months; woolly, 33 months; marmoset, 31-38 months; and one species of old world monkeys, the rhesus with an incubation period of 8 1/2 years. The virus passes through a Millipore filter of 450 nm and 220 nm but thusfar has been retained by filters of 100 nm and smaller. The virus causes disease in animals inoculated with brain tissue by the IC or IC + IV routes but may also be transmitted to animals inoculated by multiple peripheral routes (IP,IM,SC,IV) or by the IM route only. Human brain contains at least $10^6$ infectious units per gram of tissue and in chimpanzee brain it exceeds $10^7$ infectious units per gram. Virus is apparently widely distributed in visceral tissues of affected animals and disease has been induced in chimpanzees injected with pools of spleen, lymph node and kidney tissues. The virus is stable at 85°C for 30 minutes, can be lyophylized and stored at -20°C and remains fully infectious when stored at -70°C in whole tissues or suspensions of tissues. The virus persists _in vitro_ in explanted cultures of kuru affected chimpanzee brain maintained at 37°C for at least 70 days but is not associated with CPE; it does, however, retain the capacity to induce fatal disease in primates following incubation periods not appreciably different from that in animals inoculated with suspensions of infected tissues. To date we have not been able to elicit a specific, direct and indirect, antigen-antibody mechanism in naturally affected humans or experimentally infected animals, nor has it yet been possible to experimentally induce detectable levels of antibody in animals hyperimmunized with multiple doses of highly infectious tissues.

_Transmissible dementia of the Creutzfeldt-Jakob type_: The successful transmission of kuru subsequently has led to the discovery that the Creutzfeldt-Jakob type of presenile dementia is also transmissible to the chimpanzee after asymptomatic incubation periods that have ranged from 11-26 months. The disease can also be transmitted to 5 species of new world monkeys (spider, 21-37 months; squirrel, 13-25 months; capuchin, 29-39 months; woolly, 21 months; marmoset, 43 months); in addition, the disease has also been transmitted to 3 species of old world monkeys (bushbaby, 16 months; mangabey, 41 months, African green, 33-47 months) and, more recently, has now been transmitted to the domestic cat in which the incubation period was 30 months. The disease, like kuru, can be induced in animals inoculated peripherally only (IP,SC,IM) and although the virus is serially transmissible there has been no appreciable reduction in the incubation period. The virus passes through 450 nm and 220 nm Millipore filters but is held back by 100 nm and smaller filters. The virus persists and remains fully infectious for animals in explant cultures of human brain maintained _in vitro_ in third serial subculture for over 255 days.

Isolations have been made from 20 human cases. Most cases have been sporadic in occurrence; however, we have recently isolated the virus from 2 well-documented cases of the familial type thus indicating that this apparently genetically determined form of the disease is transmissible. In this context, familial cases of Alzheimer's disease appear to be more common. However, in some of these families the medical history of some of the individuals suggest C-J disease rather than Alzheimer's disease. From one such family we have recently transmitted the disease and the resulting pathology, restricted to the gray matter of the brain, is that of spongiform encephalopathy. It is significant to note that in the 10 year history of that phase of the program dealing with the isolation and characterization of viruses from human and animal CNS diseases there has been no evidence of animal to animal spread of the diseases by contact or other exogenous means within the more than 3,000 primates and over 30,000 small animals used to date.

_Focal movement disorders in rhesus monkeys following experimental infection with a strain of tick-borne encephalitis virus_: (D.M. Asher, M.D.) As described in previous reports we are attempting to define the mechanisms by which intracerebral inoculation with some strains of tick-borne encephalitis virus produces chronic movement disorders in rhesus monkeys, as a model for our studies of chronic encephalitis with focal epilepsy in humans.

To date nineteen monkeys have been inoculated intracerebrally with Vasil'chenko strain of tick-borne encephalitis virus from one to two and a half years ago. Five developed writhing choreoathetotic tremors of one hand and forearm, one with involvement of the toes of one foot as well. Another developed major motor seizures. Two animals with choreoathetotic movements (one of which had returned to normal within a year after onset), one normal animal without sequela after uneventful recovery from mild acute encephalitis, one with acute encephalitis and a control monkey inoculated with normal mouse brain were sacrificed by perfusion; histopathological changes in their brains are under study. Brain, CSF and kidney from seven monkeys (one of which had recovered from choreoathetosis about a year after onset) were examined for the presence of virus by serial passages in suckling mice; no virus has been isolated. Tissue cultures have been established from many organs including several areas of brain. Antibody responses to tick-borne encephalitis virus did not appear to differ between monkeys with and without choreoathetosis.

Remaining monkeys will be sacrificed, virus isolations attempted, and tissue cultures established. Selected tissues and tissue cultures will be examined for presence of tick-borne encephalitis virus antigen by fluorescent antibody technique.

Tissue from human patients with chronic focal epilepsy, spastic hemiparesis and dementia, and with a rapidly progressive fatal course, have been received, explanted successfully, and passaged into mice and tissue cultures; no agents have as yet been recovered. Attempts are being made to activate latent viruses in these cultures. Patients sera are being examined for antibodies to known arboviruses.

Tissue and cell culture studies: (N. Rogers, L. Gilbert, M. Lewis, J. Hooks, D. Asher, C. Gibbs, Jr., D.C. Gajdusek) Major emphasis has continued to be placed on the development and utilization of tissue and cell culture techniques in the study of slow, latent and temperate viruses. Such studies designed to isolate, identify and characterize viruses from human and animal tissues obtained at surgical biopsy and early autopsy from patients with chronic subacute degenerative diseases naturally acquired or experimentally induced have been most rewarding. During the period covered by this report additional data has been collected on the persistence of the viruses of kuru and C-J disease in in vitro explant cultures of brain tissue from naturally affected humans and experimentally infected animals; further, we have recently demonstrated that these viruses can, in the absence of observable CPE, be serially passed from infected explant cultures to BSC-1, Flow 3000 and HEK cell lines. These experiments suggest, for the first time, in vitro replication.

During the period covered by this report the following isolations have been made from non-human primate tissues in vitro: A) foamy viruses - spider monkeys (6); capuchin monkeys (14); squirrel monkeys (5); rhesus monkeys (7); chimpanzees (30); mangabey (2); pigtail macque (1). B) herpes-like viruses - spider monkeys (7); capuchin monkeys (7); squirrel monkeys (5); African green monkeys (1). Many of these isolates have been typed and data are reported in other sub-sections of this report; still others remain, as yet, unidentified.

Cell lines have been established on a variety of tissues obtained at necropsy from the following animals: chimpanzees (12); capuchins (5); squirrel (7); spider (4); African green (3); mangabey (1). The majority of these animals were killed in advanced clinical stage of kuru or C-J disease; however, cell cultures have been established with tissues from animals dying with transmissible papulosis atrophicans of Degos and familial Alzheimer's disease.

Cell lines have also been established from human brain and visceral tissues obtained at surgical biopsy and early autopsy from human patients with: C-J disease of the sporadic and familial types; familial Alzheimer's disease; progressive dementia of unknown etiology; astrocytomas; polymyositis; ALS; MS; chronic encephalitis; PML; and a number of other CNS and systemic diseases of unknown etiology. Papova virus types have been identified in the PML cases by use of the EM-hemagglutination test.

Simian foamy viruses: (J. Hooks, N. Rogers, M. Lewis) The simian foamy viruses are RNA viruses associated with a reverse transcriptase. Although they belong to the oncornavirus group, these viruses have not been shown to induce clinical disease in their natural host or in experimental animals. The viruses have a widespread distribution in all of the primates tested and preliminary evidence indicates that the foamy virus isolates from capuchin and spider monkeys are new foamy viruses. The presence of virus in the buffy coat from a spider monkey, and in the lymphocyte cultures from a capuchin monkey could explain the mechanism of viral dispersal within the natural host. The mechanism of virus spread within a population of primates is not fully understood. Since the foamy viruses have been isolated from throat washings and not from urine or feces, it would appear that horizontal transmission could take place by the respiratory route. Vertical virus transmission has been demonstrated by the isolation of a foamy virus from a rhesus monkey cervix, placenta and the kidney of the fetus.

The spider monkey isolate has been designated S140 foamy virus type 8. Cytopathic effect was observed both in the brain culture and in human embryonic kidney (HEK) cells. Neutralizing antibody was present in the spider monkey sera from whom the isolation was made. The virus is inhibited by BrdU (20 ug/ml), contains an RNA dependent DNA polymerase and has an infectivity peak at 1.15 gm/cm in a sucrose density gradient. The virus passes through a 220 nm but not through a 100 nm Millipore filter, is chloroform sensitive and is inactivated at 50°C for 30 minutes. Hemagglutinating and hemadsorption activity was not noted with a variety of erythrocytes.

By electron microscopy, virus particles are observed only within the cytoplasm. Aggreates of ring-shaped nucleoid particles measuring 45-55 nm in diameter are observed within the cytoplasm. Single-membrane-enveloped virions measuring 80-85 nm in diameter are often collected within the cytoplasmic vacuoles of smooth endoplasmic reticulum. Double membrane-enveloped virions appear to derive from the nucleoids which are enclosed by tubular membrane profiles. The tubular membrane profile engulf the nucleoid and are seen to enclose an individual particle to form a complete virion, 105-115 nm in diameter.

The virus is not neutralized by sera prepared against prototype viruses, including simian foamy virus types 1 through 7, Mason-Pfizer monkey virus (MP-MV), bovine syncytial and measles viruses. Sera from a rabbit hyperimmunized with the isolate and sera from spider monkeys were devoid of neutralizing antibody to simian foamy virus types 1 through 7. Neutralizing antibody is present in 19 of 31 spider monkey sera tested, whereas, no neutralizing antibody was detected to the virus in sera from 16 humans, 9 rhesus monkeys, 10 chimpanzees, 8 guinea pigs, 11 rabbits and 5 goats.

The biochemical and morphological characteristics of this group of viruses places them in the oncornavirus group. The modes of expression of the reverse transcriptase viruses in their natural hosts requires further study. Knowledge of patterns of antibody to these viruses in other primate species and man is essential for the better understanding of the epidemiology and biology of these syncytial forming viruses.

Studies on transformed human brain tissue in vitro and characterization of associated virus: (J. Hooks, Ph.D., C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D.) In previous reports we have described the in vitro transformation of human brain cell cultures, derived from a patient with Creutzfeldt-Jakob disease. Virus particles morphologically akin to oncogenic RNA viruses are present in the transformed cells. Our objective has been to further characterize the isolated virus, attempt to transmit C-J disease with both 10% suspension of the human brain and the transformed cell line, and finally to determine the relationship of this virus with human disease.

To date, 22 months after inoculation, 10 percent brain suspensions and suspension of the transformed S.G. cell line have not yet induced Creutzfeldt-Jakob disease in primates.

The virus particles present in the human brain cell cultures do not fit the morphological definitions of either type C or type B particles; they do resemble Mason-Pfizer monkey virus (MP-MV) described by Chopra and Mason. In collaborative studies, we have transmitted the S.G. virus to a human sarcoma cell line and demonstrated in these cells an RNA-dependent DNA polymerase that immunologically cross-reacts with the DNA polymerase of MP-MV. The S.G. transformed cell line does not contain reverse transcriptase activity. Also, the protein patterns of the S.G. virus and MP-MV, as analyzed by polyacrylamide gel electrophoresis, are similar in the number and relative size of peaks. Further studies are required to determine if the S.G. virus and MP-MV are separate isolates of a similar virus. Additional isolations of these viruses will be necessary to resolve the question.

Isolation and characterization of CMV from urine of chimpanzees: (D. Asher, M.D., J. Hooks, Ph.D., C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D.) A herpes virus (cytomegalo-type) cytopathic effect was induced in MA-184 cell lines inoculated with urine from chimpanzees A173 and A226. Urine from one additional chimpanzee (A217) has been inoculated into HEK and MA-184 cell lines.

The virus isolates will be examined by acridine orange staining, electron microscopy and sensitivity to BrdU will be determined. The relationship of these isolates to the known human and monkey cytomegalo viruses will be determined by neutralization and complement fixation procedures. A serological survey will be initiated in order to determine the distribution of the virus in chimpanzees, man and subhuman primate species.

Persistent asymptomatic cytomegalovirus infections of healthy rhesus monkeys: (D. Asher, M.D.) Chronic asymptomatic viruria of monkeys with cytomegalovirus is similar to that of man and simian infections may be useful models of human disease.

A group of healthy young rhesus monkeys were examined in 1968 and again in 1972. Most monkeys had cytomegalovirus in the urine at both times. Rhesus cytomegalovirus has not previously been isolated; although similar to cytomegalovirus of the African green monkey in biological properties the rhesus virus was not neutralized by sera which neutralize the African green virus, nor by antisera to human cytomegalovirus. The complement fixation test showed that rhesus, African green and human cytomegaloviruses shared some antigenic determinants. When a rhesus monkey chronically infected with rhesus cytomegalovirus was inoculated with human cytomegalovirus no neutralizing antibody to the human virus appeared, but there appeared to be an increase in neutralizing antibody to the homologous rhesus virus.

The antigenic relationship of rhesus cytomegalovirus to the newly-isolated chimpanzee cytomegalovirus will be studied. Humoral and cellular responses of rhesus monkeys chronically infected with rhesus cytomegalovirus to inoculation with homologous and heterologous cytomegaloviruses will be examined.

Characterization and identification of new herpes viruses from explant cultures of tissues from subhuman primates: (M. Lewis, B.S., N. Rogers, B.S., J. Hooks, Ph.D., C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D.) In the course of routine tissue culture work in our laboratory, we have observed herpes-like CPE in several of our simian cultures. Upon inoculation into stationary tube cultures of MA-184 these isolates produce characteristic herpes-like CPE. Thusfar, isolations have been made from the following: capuchins - AP18 brain cortex; AL5 spleen and lymph node; AL14 spleen; AL3 spleen; AL37 spleen and skin. Spider monkeys - S232 brain, lymph node and spleen. Squirrel monkeys - 0142 spleen and kidney; 0157 spleen and kidney; 039 spleen. African green monkey 680Z kidney. To date, however, only two of these agents have been characterized i.e. AP18 cortex and AL5 spleen isolates.

In fibroblastic cells (human skin and muscle, human foreskin, WI38, Flow 3000, and squirrel monkey fetal lung) the isolate induces a typical herpes virus cytopathic effect (CPE) consisting of rounded and ballooned cells. The capuchin herpes-like virus does not induce CPE in human embryonic kidney, owl monkey kidney or squirrel monkey kidney cells. By electron microscopy, the virion is observed both in the nucleus and cytoplasm and morphologically resembles a herpes virus. Acridine orange staining of AP18 infected WI38 cell cultures revealed small DNA containing nuclear inclusion bodies. The virus is chloroform sensitive, is inactivated at 56°C for 30 minutes, is extremely sensitive to freezing and thawing and lyophilization, and passes through a 220 nm but not through a 100 nm Millipore filter. Hemagglutination and hemadsorption studies were negative for a variety of erythrocytes. The isolate appears to be deficient in thymidine kinase since it is not inhibited by BUDR (40 mg/ml) but is inhibited by FUDR ($10^{-2}$M concentration). The herpes-like virus did not induce clinical disease or abnormalities in newborn mice, embryonating hen's eggs and a capuchin monkey.

Neutralizing antibody was present in the serum of the capuchin monkey from whom the isolation was made. The virus is not neutralized by 15 different prototype herpes-virus antisera nor has it been possible to demonstrate any neutralizing antibody in the sera of guinea pigs, rabbits and a capuchin monkey hyperimmunized with the agent. Neutralizing antibody to the isolate was demonstrated in 7 of 16 capuchin monkey sera tested whereas no neutralizing antibody was detected in the serum of 16 humans, 6 rhesus monkeys, 5 squirrel monkeys, 10 spider monkeys and 15 chimpanzees tested.

There has been a renewed interest in herpes viruses due to the implication of 2 simian herpes viruses, H. Saimiri and H. Ateles, in oncogenesis in primates.

Studies on the characterization and nature of the viruses of scrapie, transmissible mink encephalopathy and visna diseases: (C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D., M.P. Sulima, A.E. Bacote, H. Amyx, D.V.M.) As a disease entity, scrapie manifests clinical signs and neuropathological lesions remarkedly similar to those observed in human patients with kuru and Creutzfeldt-Jakob disease. Scrapie is caused by a serially transmissible, atypical virus which has a size of approximately 20 to 30 nm. The virus is unusually resistant to inactivation by ultraviolet light, formalin, heat, beta-propriolactone, but is sensitive to the action of phenol and diethyl ether. In cesium chloride it has a buoyant density of about 1.32. The virus induces a subacute progressive degenerative disease of the nervous system in sheep and goats naturally infected and produces the same fatal disease when injected in small amounts into mice, rats, hamsters, gerbils, voles, and mink. Of significance is our recent report on the transmission of scrapie to the cynomolgus monkey after an asymptomatic incubation period of five years and five months, the first successful transmission of this virus to a subhuman primate; additional transmissions of several strains of scrapie have now been accomplished in squirrel monkeys (incubation period 14 months). The squirrel monkey thus becomes the first experimental host susceptible to each of the prototype spongiform encephalopathies. This further suggests the possibility that the original hosts and perhaps even the reservoirs of kuru and Creutzfeldt-Jakob disease may be animals. Ultracentrifuge zonal gradient studies indicate maximum peaks of infectivity associated with peak zones of nucleic acid in brain tissue suspensions; these data strongly suggest that scrapie is associated with small fragments of nucleic acid (probably double stranded DNA) that is tightly bound to plasma membrane. Using the technique of Diener, we are attempting to isolate small molecules of naked nucleic acid from scrapie infected mouse brain and spleen. Continued attempts to demonstrate specific antigen-antibody reactions to scrapie remain unsuccessful. The immune mechanism of the scrapie infected host remains functional and without alteration on both the humoral and cellular levels. Administration of large doses of interferon, poly I-poly C, x-irradiation and antilymphocytic serum have all failed to interrupt or alter the onset or course of this fatal disease of animals. An extensive electron microscopic search for virus in the fractions of brain suspension obtained by density gradient banding the agent in zonal ultracentrifuge have revealed maximum infectivity is present in those fractions which are composed almost entirely of plasma membrane. Although we can see no consistent evidence of fully formed mature virions, we are certain that degenerative processes seen must represent the effect of virus production and maturation, e.g. alteration of the plasma membranes of cells resulting in fragmentation of the unit membrane and the filling of intraneuronal vacuoles with this membraneous material; in vivo fusion of astrocytes with astrocytes and astrocytes with neurons, further suggesting alteration of the membrane.

Clinical manifestations of experimental kuru and Creutzfeldt-Jakob disease in the chimpanzee: (H. Amyx, D.V.M., D. Asher, M.D., C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D.) Careful observations of clinical course, electroencephalograms, blood and spinal fluid findings allow better comparison of the experimental conditions with natural human diseases and correlations of clinical manifestations with histopathological changes.

Over forty chimpanzees with experimental kuru have been studied. All had signs of diffuse brain disease with the earliest and most severe evidence of degeneration of cerebral cortex followed by less prominent cerebellar and basal ganglia involvement. Signs of disease in medulla and spinal cord were less common and tended to appear only late in its course. There was no disturbance of function of peripheral nerves or of muscles nor of any primary disease outside the central nervous system. Electroencephalograms were diffusely abnormal. Spinal fluid proteins rose slightly but significantly late in disease. There were no consistent or significant primary changes in blood chemistries or hematologic parameters. The clinical picture of experimental kuru in the chimpanzee correlates well with histopathological findings and is generally similar to human disease from which it differs in having relatively more severe cortical and less severe cerebellar involvement. Experimental Creutzfeldt-Jakob disease in the chimpanzee resembles kuru but has a shorter incubation period, more rapid clinical course, and prominent myoclonic jerks, paresis of limbs and sometimes somnolence not found in experimental kuru.

Electroencephalographic, spinal fluid and blood studies of chimpanzees with experimental Creutzfeldt-Jakob disease are in progress.

Animal management and intercurrent diseases in subhuman primates on long-term studies of slow infections: (H. Amyx, D.V.M., D. Asher, M.D., C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D.) A wide variety of laboratory animals are utilized at the NINDS virus laboratory at P.W.R.C.: rabbits, guinea pigs, hamsters, mice, rats, ferrets, mink, sheep, cats, and non-human primates. The non-human primates number 385 new and old world monkeys and 75 chimpanzees.

Major medical problems in the primate colony arise from two sources: traumatic injuries and infectious diseases. Lesions resulting from group and pair fighting account for almost all of the surgical procedures at our laboratory, but for only several fatalities in the past year.

Infectious diseases have been the primary cause of primate mortality. Amebiasis due to Endamoeba histolytica has accounted for most fatalities in spider monkeys. This species appears to be extremely susceptible to visceral complications from Endamoeba. Three cases of fatal balantidiasis (Balantidium coli), also in spider monkeys, were recorded. Histologic lesions present in these cases have been described rarely in balantidial infections, which are generally mild in primates and man.

Shigellosis strikes sporadically, but generally in the hottest part of the summer, in the outdoor cages. Shigella dysenteriae and sonnei were recovered from clinical cases of dysentery this past summer.

Helminth parasites, especially pinworms and hookworms, are a constant problem but rarely result in severe disease. However, severe parasitism with the Trematode Athesmia foxi was found in a number of capuchins (Cebus albifrons).

Although herpes viruses are isolated regularly from most primates in the colony, only one animal has ever shown histologic evidence of herpes virus infections.

Studies on the polymorphonuclear depressing factor in multiple sclerosis and other slow infections of the nervous system: (P. Brown, M.D., D.C. Gajdusek, M.D., C.J. Gibbs, Jr., Ph.D.) Carp et al. have reported that suspensions of brain tissue from human patients with multiple sclerosis and from mice with scrapie when injected into mice induces an alteration in the percent polymorphonuclear neutrophils in clinically normal mice after inoculation. These investigators further reported recovery of an agent that could be serially transmitted in mice, was self replicating and has a size of 25-50 nm. In an effort to confirm these findings a series of experiments were conducted in several lines of inbred and randomly bred mice inoculated with human derived MS brain, including the Carp inoculum, and mouse derived scrapie viruses. Complete blood counts including differential leukocyte counts were performed. We have been unable to confirm the finding of decreased percentage of polymorphonuclear neutrophils in mouse peripheral blood after inoculation with materials from multiple sclerosis patients and materials from mice with scrapie.

Development of serological and immunological test system for use in the study of slow infections of the CNS: (D.C. Gajdusek, M.D., C.J. Gibbs, Jr., Ph.D., P. Brown, M.D., F. Cathala, M.D., R. Benfante.) During the period covered by this report serum and CSF specimens from humans with kuru, C-J diseases, Alzheimer's disease, chronic encephalitis with myoclonus and focal epilepsy, and a wide variety of other subacute chronic CNS diseases as well as pre-and post-serum and CSF from animals with experimentally induced diseases have been tested by direct and indirect complement fixation, mixed hemagglutination, hemadsorption, direct and indirect FA, and, for antibody to lytic and non-lytic neoantigens at cell surface loci in cell lines derived from human and animal CNS tissues. To date no specific antigen-antibody systems for kuru, transmissible dementia of the C-J type, or scrapie have been developed.

Immune responsiveness of multiple sclerosis patients to established viral antigens by detection of specific antibodies in serum and cerebrospinal fluids collected serially during remissions and exacerbations: (P. Brown, M.D., F. Cathala, M.D., C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D.) Studies to establish infection as the etiology of some forms of multiple sclerosis have been continued and expanded during the past year. Recent findings in our laboratory have confirmed the earlier observation that a significant number of MS patients have antibody to measles virus in their CSF, in sharp contrast to non-MS control humans. A second group of serum and CSF specimens from additional humans with MS had antibody to vaccinia virus, as previously reported by Kempe et al. in Colorado. Virus particles morphologically similar to myxovirus-paramyxovirus nucleocapsids have been seen by EM in plaque and periplaque areas of brain from humans with MS. ter Muelen et al., have reported the isolation of "cold-mutant" strain of para-influenza type 1 virus from 2 cases of MS. However, we have not been able to demonstrate significant levels of antibody to para-influenza 1 virus in our collection of serum/CSF specimens from MS patients. Our data does, however, support our earlier hypothesis that some types of MS have a viral etiology and, in at least some instances, a defective measles virus and perhaps vaccinia virus are involved. It thus appears that MS may well be a disease of multi-virus etiology. A major team study to elicit the cause of MS, to develop an experimental model to study the disease and to characterize the agents involved is well underway.

Significance to biomedical research and the program of the institute: The mission assigned this laboratory in 1962 was to demonstrate that subacute chronic progressive degenerative diseases of the nervous system of man may have infection as their etiology. The data presented in this report conclusively demonstrate that the subacute degenerative encephalopathies, kuru, a heredofamilial disease of man, and Creutzfeldt-Jakob disease, a presenile dementia of man, are both caused by filterable infectious agents which behave like viruses in biological, chemical and physical properties. These studies have further demonstrated, for the first time, that scrapie, a natural encephalopathy of sheep and goats which closely resembles kuru and Creutzfeldt-Jakob disease in clinical course and histopathology, can infect sub-human primates, the cynomolgus monkey and the squirrel monkey. It is possible that the original hosts and perhaps even the reservoirs of kuru and C-J disease may be animals, and emphasize the fact that the scrapie virus with its worldwide distribution may not be inocuous for man. It is worthy of note that a wide variety of "clinical and histopathological types" of Creutzfeldt-Jakob disease, including those described as subacute spongiform encephalopathy, Nevin-Jones disease, Brownell-Oppenheimer disease, as well as the amyotrophic form as originally described by Creutzfeldt and Jakob, have in every instance induced remarkedly similar fatal clinical disease and histological lesions in experimentally inoculated animals with incubation periods of one year or less and rapid clinical courses of only one to three months duration. In addition, we have demonstrated that familial cases of Creutzfeldt-Jakob disease are also transmissible. Our reports on the transmissibility of C-J disease, previously thought to be a rare disease, have in recent months resulted in the acquisition of brain specimens from more than one hundred new cases--more than doubling the world's previously reported cases. These findings support our hypothesis that other encephalopathies, including the so-called presenile and perhaps even senile dementias of man, such as Alzheimer's and Pick's diseases, as well as diseases such as multiple sclerosis, amyotrophic lateral sclerosis, Huntington's chorea, Alper's disease, progressive supranuclear palsy, chronic encephalitis with focal epilepsy and myoclonus may be caused by similar agents. More definitive information on the nature of the agents causing kuru and Creutzfeldt-Jakob disease will undoubtedly lead to eliciting the causative agents of these other diseases. The successful transmission of kuru to rhesus monkeys with an 8 1/2 year incubation period draws attention to the possibility that sub-human primates inoculated with other neurological diseases of man may yet manifest clinical signs of disease associated with exceedingly long incubation periods. Our recent transmission of kuru to marmosets and adaptation of the virus in this animal, resulting in an appreciable lowering of the incubation period from 36 months on primary passage to 42 days on serial passage, provides the most sensitive test model to date for efficiently characterizing and determining the nature of kuru. Indeed, these studies have formed the basis of increasing interest and worldwide investigations in the relation between viruses and chronic degenerative diseases of the brain. The isolation of kuru virus has been followed by the demonstration that defective measles virus is associated with subacute sclerosing panencephalities of children and young adults; the demonstration of a papova virus etiology for progressive multifocal leukoencephalopathy and the isolation of SV40 related virus from the brains of at least two patients with this disease; and, the demonstration of virus inclusion bodies in patients with chronic epilepsy or epilepsia partialis continua. With our recent demonstration in cell cultures from the brain of a patient with Creutzfeldt-Jakob disease a defective virus which in some respects resembles a "conventional" tumor virus we have opened important and new questions about possible synergistic relationships between different latent infectious agents causing both degenerative encephalopathies and malignant neoplasms of the brain.

Proposed course: The study of slow, latent and temperate virus infections of man and animals, with particular reference to diseases of the nervous system, but also including chronic and persistent infections due to latent, masked, persistent and infectious agents will be continued along the lines described in this and previous reports. We shall continue our attempts to transmit additional neurological diseases to suitable laboratory hosts and to isolate and characterize the etiological agents of these diseases. In an effort to arrive at the definitive nature of the viruses of kuru, Creutzfeldt-Jakob disease and scrapie, we shall continue to characterize their biological chemical and physical properties. Preliminary studies have already been initiated in the area of chemo-therapeutic and immuno-prophylactic measures that may prove effective in preventing or arresting these diseases in experimental animals. Continued emphasis will be placed on the in vitro growth of human and animal tissues in attempts to isolate latent, persistent, chronic, masked viruses which may well be sharing an endosymbiotic relationship with the donor host until some exogenous stress factor activates their pathogenicity in vivo. Since the viruses of kuru, C-J disease and scrapie are now known to persist and probably replicate in vitro without lysing the cells, these cultures will be studied in greater detail by electron microscopy, cytogenetic techniques, immunologically, for the presence of cell surface antigens and their altered metabolic processes determined by incorporation of $H^3$ uridine, $H^3$ thymidine and $C^{14}$ glucose. Infectious agents isolated from human and animal tissues obtained at surgical biopsy and early autopsy will be characterized, identified and classified, and by seroepidemiological techniques we will determine their distribution and significance in human and animal populations. We shall determine their etiological significance in relation to the disease under study. Major emphasis will continue to be placed on a comprehensive study of the human brain transformed cell line developed in this laboratory from a patient with C-J disease. These studies, in collaboration with the National Cancer Institute, will include purification of the virus and study of the oncogenic, tumorigenic, immunological, chemical and physical properties. Extensive field work will be continued on kuru and related diseases, particularly focusing on the epidemiology and natural reservoirs since cases are still continuing to occur.

Serial No. NDS (IR) - 65 OD 1282
1. Intramural Research
2. Central Nervous System Studies Laboratory
3. Bethesda, Maryland

PHS - NIH
Project Report
July 1, 1972 through June 30, 1973

Project Title: Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures

Sub-Project I: Study of the developmental patterning of the human nervous system (cybernetics of human development).

Sub-Project II: Human evolutionary studies in isolated primitive groups.

Sub-Project III: Studies of isolated Micronesian populations.

Sub-Project IV: Studies of isolated New Guinea populations.

Sub-Project V: Studies of isolated New Hebrides and Solomon Islands populations:
Research Vessel ALPHA HELIX Expedition. September 17 to November 18, 1972.

Sub-Project VI: Studies of Australian Aborigines.

Sub-Project VII: Studies of Central and South American Indians.

Sub-Project VIII: Developmental, genetic and disease patterns in primitive populations of Asia, Africa and Polynesia.

Sub-Project IX: Experimental developmental neuropediatrics in infantile programming: an empirical approach to the language of information input into the nervous system.

Sub-Project X: Ciphers and notation for the coding of sensory data for neurological information processing.

Sub-Project XI: Racial distribution and neuroanatomic variations in the structure of the human brain.

Principal Investigators: D. Carleton Gajdusek, M.D.
Clarence J. Gibbs, Ph. D.
Paul Brown, M.D.

Other Investigators: Michael Alpers, M.D., David M. Asher, M.D., Mint Basnight, M.S., Richard Benfante, M.A., Richard A. Ferber, M.D., Peter Fetchko, M.A., Larry Frye, Ph.D., Ralph Garruto, Ph.D., Jeffrey Kroeber, Ivan Mbaginta'o, Judith Meyer, Steven Ono, Nancy Rogers, M.S., Donald Rubinstein, M.A., E. Richard Sorenson, Ph.D., Nancy VanWyck, B.S., Stephen Wiesenfeld, M.D., Vincent Zigas, M.D.

Cooperating Units: Timothy Asch, Dept. of Anthropology, Brandeis University, Waltham; Steven Brown, M.D., School of Public Health, University of California, Berkeley; N. Chagnon, Dept. of Anthropology, University of Michigan, Ann Arbor; Edwin Cook, Dept. of Anthropology, University of California, Davis; Dr. Kenneth Dresser, Father F. Trenkenschuh, and Father D. Gallus, West Irian, Indonesia; Rev. Hartmut Gericke, Goroka, New Guinea; Dr. T. Gerungen, Public Health Department, West Irian; Dr. Gordon Gibson and Dr. Saul Riesenberg, Smithsonian Institution, Washington, D.C.; Dr. Maurice Godelier and Dr. Jean Guiart, Sorbonne; Dr. Roger Greenough, Dr. P. deCarfort, and Dr. R.C. Walsh, New Hebrides; Mr. and Mrs. Jablonka, Columbia University, New York; Mr. and Mrs. P. Kennedy, Dept. of Anthropology, University of Buffalo, New York; Thomas Kiefer, Dept. of Anthropology, Brown University, Providence; Dr. R. Kirk, Dr. N.M. Blake and Dr. K. Omoto, Dept. of Human Biology, Australian National University, Canberra; Dr. David Kitchin, Columbia-Presbyterian Medical Center, New York; Dr. Peter Kunstadter, Dept. of Preventive Medicine, University of Washington, Seattle; Dr. H. Lehmann, University of Cambridge, England; W. Leyshon and Dr. K.S. Brown, National Institute of Dental Research, NIH; Richard Lloyd, Summer Institute of Linguistics, Papua New Guinea; Alan Lomax, Columbia University, New York; Dr. James MacGregor and Dr. B. Eyers, Public Health Department, British Solomon Islands; Dr. Paul MacLean, Mental Health Institute, NIH; Dr. Robert MacLennan, International Agency for Cancer Research, Lyon, France; Margaret Mead, American Museum of Natural History, New York; Dr. J.A. Morris, Bureau of Biologics, Bethesda; Chris Plato, NICHD, NIH; Dr. Roger Rodrigue, Springfield, Pa.; Dr. Frank Schofield, Kenyatta National Hospital, Nairobi; Dr. Theodore Schwartz, Dept. of Anthropology, University of California, Los Angeles; Dr. John Sheridan, Walter and Eliza Hall Institute, Melbourne; Dr. R.T. Simmons and Dr. J.J. Graydon, Commonwealth Serum Laboratories, Melbourne; Dr. Malcolm Simons, W.H.O. Immunology Centre, Singapore; Bishop Alphonse Sowada, West Irian; Dr. Neville Stanley, University of Western Australia, Perth; Dr. J. Sulianti-Sarosa, Department of Public Health, Djakarta, Indonesia; Dr. L. Vogel, Medical Research Centre, Nairobi; Dr. S.A. Wurm, Australian National University, Canberra; Dr. P. Yakovlev and Dr. H. Hamlin, Walter Fernald School, Waverly, Mass., Dr. F. Cathala, Hopital Salpetriere, Paris; Dr. Raymond Roos, Johns Hopkins University Hospital, Baltimore; Dr. David Lang, Duke University Medical School, Durham; Dr. Keerti Shah, Johns Hopkins University Hospital.

Man Years

| | |
|---|---|
| Total: | 38-1/3 |
| Professional: | 12-1/3 |
| Others: | 26 |

The Projects (I through XI) listed herein, and their related studies, itemized in the Project Reports of previous years, have continued throughout the year and are reflected in the extensive list of publications noted herein. The laboratory studies on human biology, genetics and associated molecular biology, immunology, virology and biochemistry, are all directed at solving problems which have been chosen from small isolated bands still living in the primitive situation, in which these problems may be more appropriately studied than in larger civilized societies. Our studies in Micronesia, in New Hebrides and the British Solomon Islands were greatly expanded during this year as a result of the R/V Alpha Helix expedition into those islands; a prospectus of the studies envisaged as resulting from the expedition is herein enclosed.

Serial No. NDS(IR) - 65 OD 1282

Sub-Project V: Studies of isolated New Hebrides and Solomon Islands populations:
Research Vessel ALPHA HELIX Expedition. September 17 to November 18, 1972.

## Medical and Population Genetic Survey of the Banks and Torres Islands of the New Hebrides and the Southern Islands of the British Solomon Islands Protectorate

Chief Scientist: D. C. Gajdusek, M.D.

Cruise Participants:

D. Bowdin, M.D., Public Health Department, New Hebrides.
P.W. Brown, M.D., National Institutes of Health, Bethesda.
F. Cathala, M.D., Hopital Salpetriere, Paris, France.
R. Feinberg, University of Chicago, Chicago.
R.A. Ferber, M.D., National Institutes of Health, Bethesda.
W. Firipae, Public Health Department, Rennell Island, B.S.I.P.
J. Guiart, Ph.D., Sorbonne, Paris.
R. Lee, M.D., Public Health Department, Santa Cruz, B.S.I.P.
I. Mbaginta'o, National Institutes of Health, Bethesda.
J. Meyer, National Institutes of Health, Bethesda.
S. Reveag, Public Health Department, Mota Lava, New Hebrides.
R. Roos, M.D., Johns Hopkins University Hospital, Baltimore.
D. Rubinstein, Stanford University, Palo Alto.
W. Schneider, Scripps Institution of Oceanography, San Diego.
J. Sheridan, M.D., Walter and Eliza Hall Institute, Melbourne, Australia.

Local Collaborating Investigators:

New Hebrides

J. Barley, Captain, Condominium Customs Department, Port Vila.
P. de Carfort, M.D., Director, Condominium and French Medical Services, Port Vila.
R. Greenough, M.D., Director, British Medical Services, Port Vila.
R.C. Walsh, M.D., Patton Memorial Hospital, Presbyterian Mission, Port Vila.
K. Woodward, Assistant Secretary, British Residency, Port Vila.

British Solomon Islands Protectorate

B. Eyers, M.D., Medical Service, Honiara
J. MacGregor, M.D., Director, Public Health Department, Honiara

Other Participating Investigators:

D. Asher, M.D., National Institutes of Health, Bethesda.
M. Basnight, National Institutes of Health, Bethesda.
P. Fetchko, Peabody Museum, Salem.
R. Garruto, Ph.D., National Institutes of Health, Bethesda.
C.J. Gibbs, Jr., Ph.D., National Institutes of Health, Bethesda.
J. Hooks, Ph.D., National Institutes of Health, Bethesda.
R. Kirk, M.D., Australian National University, Canberra, Australia.

J. Kroeber, National Institutes of Health, Bethesda.
D. Lang, M.D., Duke University Medical School, Durham.
L.H. Miller, M.D., National Institutes of Health, Bethesda.
G. Pacheco, Ph.D., National Institutes of Health, Bethesda.
C. Plato, National Institutes of Health, Bethesda.
N.G. Rogers, National Institutes of Health, Bethesda.
R. Saldino, M.D., National Institutes of Health, Bethesda.
K. Shah, M.D., Johns Hopkins University Hospital, Baltimore.
M. Simons, M.D., University of Singapore, Malaya.
S. Wiesenfeld, M.D., National Institutes of Health, Bethesda.

Islands Studied on the R/V Alpha Helix Expedition to the Banks and Torres Islands, New Hebrides, and the Southern Solomon Islands:

New Hebrides

- Banks Islands
  - Mera Lava (Meralav)
  - Merig
  - Gaua
  - Vanua Lava
    - Offshore Islands:
      - Ravenga
      - Pakea
      - Niwula
  - Mota
  - Mota Lava
  - Reef Islands:
    - Rowa
    - Sana
  - Ureparapara

- Torres Islands
  - Toga
  - Loh
    - Offshore Islands:
      - Linoua
  - Tegua
    - Offshore Island:
      - Metoma
  - Hiu

British Solomon Islands Protectorate

- Eastern District

- Santa Cruz Islands
  - Vanikoro Islands:
    - Vanikoro
    - Tevai

Utupua
Ndeni

Reef Islands
Tinakula Island
Nupani
Nalogo
Nukapu
Motema
Banga Ndeni
Banga Netepa
Lomlom
Fenualoa
Pileni
Sand
Nifiloli
Duff Islands

Obelisk Islands
Treasurer Islands
Disappointment
Bass Islands

Polynesian Outliers
Tikopia
Anuta
Fataka

Central District
Polynesian Outliers:

Rennell
Bellona

The purpose of the expedition was to make use of the laboratories and support facilities of the R/V Alpha Helix for a thorough medical, population genetics, and human biology survey in a series of very remote islands difficult of access because of their total lack of harbors and anchorage. These islands are all of particular interest to medicine, anthropology and human biology, because of their genetic and epidemiologic isolation, limited ecology, and the fact that they are an area of high inbreeding, and of overlap of Melanesian and Polynesian peoples. Almost all of them present difficulties in disembarkation and embarkation, and at very few of them was it possible to anchor. During much of the year, including much of the period of this expedition, the seas are rough, often making landing impossible. These facts, and the remoteness of the islands, have made medical surveys and laboratory studies of these populations impossible in the past.

The difficulties in bringing the people to or from the ship severely limited the possibility of shipboard laboratory studies of patients. Thorough medical, including neurological examinations, x-ray studies, and other laboratory tests were often difficult or impossible on board ship because of rough seas and without anchorage. Thus, such use of the ship's facilities had to be devised as would best serve the unique requirements for medical work on these small volcanic islands. Without the Alpha Helix' power supply, refrigerators, microscopes, centrifuges, incubators and liquid nitrogen, the expedition would have been impossible.

However, the purpose of the expedition was medicine and human biology, which demanded shore-based, not ship-based research, and the uses to which the ship's laboratories were put was largely that of the complex processing of biological specimens for their continued study over a period of years in the collaborating laboratories in Europe, Australia and America. Unlike many previous Alpha Helix expeditions the major laboratory data from these studies will be accumulated for over a decade and the definitive publications are not envisaged before several years of laboratory studies have been completed.

In June 1972 the Chief Scientist visited both the New Hebrides and the Solomon Islands to discuss the problems and purposes of the expedition with both administrators and medical services on these islands. He encountered enthusiasm for the expedition and received extensive hospitality; he was permitted access to census and demographic data, maps and medical reports which were essential to the work on the islands. Most important, the medical services and administrations informed the islanders of the arrival of the expedition, and the medical team was met with enthusiasm on every island. On all islands the Chiefs and the people cooperated, with patients anticipating our arrival. Interest in the extensive medical examinations was high, and no resistance was encountered to such examinations or to the collection of blood, urine, fecal, saliva and other specimens, or to the skin testing and other diagnostic precedures. The people welcomed the opportunity to present their many medical complaints to the assembled medical specialists, and were enthusiastic and careful in providing the necessary anamnestic and general background information.

The nature and purpose of the studies were explained to the people on these islands, particularly to the Chiefs and islanders who served in the local schools and churches. Medical findings, particularly on individual patients who required further medical attention, were brought to the attention of government medical services in both countries. On the large islands, such as Efate and Santa Cruz--not included in the concentrated medical, genetic and human biology studies--the physicians on the ship were called in as consultants by the local doctor to see many of the patients. The expedition provided all medical attention possible to those on the islands that required it, but no surgical procedures were attempted, and patients who required elective surgery were referred to the respective directors of health services.

Most of the scientific party from overseas joined the program at Port Vila and left from Honiara, B.S.I.P., or from Ponape in Micronesia, where the final laboratory work was completed and the complex packing and dispatch of incubated and refrigerated specimens was carried out. Drs. Brown and Guiart left the expedition at Santa Cruz in the Solomon Islands after the first five weeks of study, and Dr. Sheridan joined us there. Dr. Bowdin was with the expedition only in the southern part of the Banks Islands, and Dr. Reveag only in the northern Banks and Torres Islands. Dr. Lee participated in the program on Tikopia, Anuta and Santa Cruz; and Mr. Feinberg on Anuta, where he had been in residence for six months on anthropological field work; and Mr. Firipae on Rennell and Bellona Islands. Mr. Mbaginta'o first joined the expedition to participate in the final packing, cataloguing, and dispatch of specimens from Ponape. There, the chief scientist, Mr. Rubinstein and Mr. Mbaginta'o continued on the next expedition of the Alpha Helix to Pingalop Atoll with the Chief Scientist, Dr. R. Carr, where they continued the medical and human biology studies on Pingalop children and departed two weeks later from Ponape.

The islands are of particular interest because of their small size and limited ecology, with very few species of mosquitoes, ticks, or mite vectors of disease; some have no mosquito vectors for malaria. They are also a major transition area in the Pacific between malaria-affected and malaria-free islands; filariasis, similarly, varies from high to low incidence or absence. Genetically and culturally the islands are of interest in that they contain several Polynesian outliers, including proto-Polynesian populations on Rennell and Bellona Islands within Melanesia. Furthermore, from our earlier investigations, we had demonstrated a new hemoglobin variant, hemoglobin J Tongariki, in the Shepherd Islands, and also in the Banks and Torres populations. We have reason to believe this will serve as a unique genetic marker for tracing past migrations in the South Pacific. Also, in our earlier studies we had encountered extraordinarily high blood group B frequencies on Rennell and Bellona Islands, indicating a strange uniqueness in these populations which demanded further genetic analysis. Since we had described a focus of familial periodic paralysis on Tongariki Island, we specifically hunted for this disease and found none. The presence of familial goiter and cretinism on Bellona Island we had also previously noted, and investigations of this disease have been expanded.

We are heavily indebted to the French and British Commissioners in Port Vila, and the British High Commissioner and his staff in Honiara for their permission to work in their respective islands, and for the administrative and logistics assistance they rendered us. We are extremely indebted to the Chiefs of Services in the local Public Health Departments in both countries who went far out of their way to give us personal service and advice from their own experiences in the islands. They made the local documents and records available to us and continue to participate in the scientific program; thus, they have been listed as local collaborating investigators.

The various studies have been divided into three major categories: 1) Disease Patterns in Primitive Isolated Populations; 2) Population Genetic Studies in Relation to Congenital Defects and Heredofamilial Diseases in Isolated Island Populations; and 3) Epidemiological Investigation of the Demography, Kinship, Migration and Marriage Patterns of the Populations Studied; Documentation of the Ethnography, Natural History, Ecology, and Medical Procedures and Findings. These are summarized herewith, with a list of the investigators who are contributing in each study. These summaries are not contemplated papers, but, rather, fields of investigation in which one or numerous more specific publications are planned.

## Disease Patterns in Primitive Isolated Populations

I. Medical survey of the island populations.

Investigators:
1. D.C. Gajdusek, M.D.
2. P.W. Brown, M.D.
3. R.A. Ferber, M.D.
4. R. Roos, M.D.
5. F. Cathala, M.D.
6. J. Sheridan, M.D.

II. Neurological disease in the Banks, Torres and Southern Solomon Is.

Investigators:
1. R. Roos, M.D.
2. F. Cathala, M.D.
3. D.C. Gajdusek, M.D.
4. P.W. Brown, M.D.
5. R.A. Ferber

III. Seroepidemiological investigations for virus, rickettsial, bacterial, mycotic, protozoal and helminthic diseases, and virus isolation studies.

Investigators:
1. D.C. Gajdusek, M.D.
2. P.W. Brown, M.D.
3. C.J. Gibbs, Jr., Ph.D.
4. N.G. Rogers
5. R. Garruto, Ph.D.
6. D. Lang, M.D.
7. K. Shah, M.D.
8. D. Asher, M.D.
9. J. Hooks, Ph.D.

IV. Survey of skin test sensitization to tuberculosis, atypical mycobacteria, coccidioidin and histoplasmin.

Investigators:
1. P.W. Brown, M.D.
2. D.C. Gajdusek, M.D.
3. R.A. Ferber, M.D.
4. R. Roos, M.D.
5. F. Cathala, M.D.
6. J. Sheridan, M.D.

V. Chest roentgenographic survey of the islands.

Investigators:
1. R.A. Ferber, M.D.
2. R.M. Saldino, M.D.
3. P.W. Brown, M.D.
4. D.C. Gajdusek, M.D.

VI. Parasitology: malarial and filarial and intestinal parasite studies.

Investigators:
1. P.W. Brown, M.D.
2. D.C. Gajdusek, M.D.
3. L.H. Miller, M.D.
4. F.A. Neva, M.D.
5. G. Pacheco, Ph.D.

Disease Patterns in Primitive Isolated Island Populations

## I. Medical Survey of the Island Populations

To determine the spectrum of diseases found on these islands, with emphasis on those of infectious, genetic or congenital etiologies, and to identify the neurological syndromes present, detailed physical examinations, supplemented as needed by laboratory investigations, were performed on over three thousand people. Examinations of entire island populations were performed on each of the smaller islands, and selected medical surveys were made on those with larger populations. Protocols included the complete neurological and ophthalmological, as well as general medical examinations. On several islands, as well as in situations where medical problems indicated the need, urinalysis, hematologic examinations of peripheral blood, thick and thin blood smears for parasite identification, x-ray and electrocardiographic study, appropriate cultures for bacteria or viruses, and intradermal skin testing with atypical mycobacterial and mycotic antigens were carried out. On all children heights and weights were recorded and identity photographs taken for use in future longitudinal growth and development studies.

## II. Neurological Disease in the Banks, Torres and Southern Solomon Islands

A survey of neurological disease was made in the Banks, Torres and Southern Solomon Islands, in order to determine the most prevalent syndromes and to search for any disease foci. The neurological diseases encountered fell into several categories: congenital abnormalities, infectious or post-infectious disease, degenerative disease, peripheral neuropathy, and cervical and lumbar spondylosis.

The congenital disease frequently resulted from definite structural damage; the examples include hydrocephalus, focal epilepsy (with secondary dystonia involving one hand), spastic paraparesis, hypotonia with hyperreflexia, club feet with hyperreflexia. At least five cretins with mental retardation were seen; two had associated severe myopathy (one cretin on Bellona had a goiter). The most common sequella of infection seen was lower motor neuron paralysis secondary to poliomyelitis. Over six cases of old poliomyelitis were seen, in each case the initial febrile illness had occurred in the setting of a presumed epidemic, since other islanders had died or been similarly affected at the same time. A number of cases of leprosy were seen, one having a classical mononeuritis. A case of herpes zoster with radicular pain was found, possibly associated with the high prevalence of varicella, presently active on the islands.

The degenerative diseases seen included two cases of parkinson's disease and two cases of an olivopontocerebellar disease syndrome. The fact that the latter two cases were sporadic, and that one patient had a history of febrile illness preceding the onset of neurological signs, may indicate that the syndrome was post-encephalitis. One islander had dementia, hyperkinesis, cerebellar signs and hyperreflexia, possibly post-encephalitis as well. Two cases of severe optic atrophy of unknown etiology were seen.

Peripheral neuropathy occurred in two individuals with a typical Charcot-Marie-Tooth clinical picture, although both cases were sporadic. Another peripheral neuropathy had a large component of posterior column disease.

One patient with peripheral neuropathy had, in association, cerebellar signs, dementia, and a long history of seizures. One of the most common complaints of the islanders was lower back pain, probably reflecting the severe spondylosis caused by physical labor. Several patients with sensory and motor radiculopathies, probably secondary to cervical spondylosis, were seen.

Certain diseases were noticeable by their absence. As has been recognized in the past, multiple sclerosis is rare in the tropics, and no case was found. Only one patient with a cerebrovascular accidnet was seen, reflecting the relative low incidence of arteriosclerotic disease and hypertension on these islands. No focus of neurological disease was seen; in particular, amyotrophic lateral sclerosis, parkinsonism-dementia and kuru-like or other tremor syndromes were not encountered, and no reports of familial periodic paralysis-like disease, such as have been found on Tongariki Island in the Shepherd Islands, New Hebrides.

## III. Seroepidemiological Investigations for Virus, Rickettsial, Bacterial, Mycotic, Protozoal and Helminthic Diseases and Virus Isolations

Sera were collected from three thousand subjects, or over 60% of the population in the islands intensively studied. On islands given our particular attention, such as Anuta and Merig, over 90% of the population was bled. Over the course of several years the sera will be investigated for a wide variety of infectious disease antibodies. Techniques of complement fixation, hemagglutination inhibition, fluorescent antibody, neutralization, indirect hemadsorption inhibition, and agglutination will be used to identify viral and rickettsial antibodies. Appropriate antibody tests for infection with various specific protozoa, bacteria, helminths, and fungi will be conducted. Age incidence of acquisition of immunity or of first contact with each microbe will be plotted to increase our understanding of the pattern of spread of infectious diseases in these population isolates.

Particular attention will be given to arbovirus infections in view of the simple ecology of these islands and the paucity of species of natural hosts and vectors which are to be found. Continuation of our studies on the mode of transmission of toxoplasmosis will be a major interest. Since none of the people have had contact with monkeys or vaccines prepared in monkey tissue cultures, the antibody survey of these populations for antibodies to the Simian viruses is of particular significance. Antibody pattern to the various papovaviruses isolated from human brain of patients with progressive multifocal leucoencephalopathy (SV 40, J.C. virus, and the related BK virus) to determine the frequency and age incidence of contact with these agents as possible clues to their epidemiology in man will be quickly determined. Similarly, in view of the importance of our understanding of the carrier state for herpes viruses in man, and the possible role of these agents in the genesis of cancer and other chronic diseases, antibody acquisition patterns for herpes hominis types 1 and 2, Epstein-Barr virus, cytomegalovirus and varicella and herpes zoster will be determined. Australian antigen and Australian antigen antibody will be determined, and also antibody response to respiratory syncytial virus and the kuru foamy viruses of man, chimpanzees, monkeys and non-primates.

A large number of specimens for virus isolation work have been collected. These include, principally, throat washings, fecal and some urine specimens; they are maintained at -70°C and are being slowly processed for the presence of viruses over the next year.

## IV. Survey of Skin Test Sensitization to Tuberculosis, Atypical Mycobacteria, Coccidioidin and Histoplasmin

Because of the high incidence of tuberculosis in the populations of some of the islands, an attempt was made to recognize all cases of tuberculosis early and to evaluate the true extent of the problem. The possibility that atypical mycobacteria may be contributing to the pattern of chronic pulmonary diseases seen was thought to be great. Intradermal skin tests, using seven mycobacterial antigens (PPD, PPD-B, PPD-F, PPD-G, PPD-S, PPD-Y and PPD-avian), coccidioidin and histoplasmin were performed on three hundred subjects of all ages on three of the islands: Mèrig, in the Banks Islands; Loh, in the Torres Islands; and Anuta, the most remote of the Polynesian outliers in the Solomon Islands. On Anuta sixty chest x-rays were taken on islanders who could reach the ship and who had been skin-tested with all nine antigens. All subjects studied had had complete physical examinations.

## V. Chest Roentgenographic Survey of the Islands

In order to determine the extent and characteristics of known and unknown pulmonary disease in the islands studied, over four hundred chest x-rays were taken by means of a portable x-ray unit aboard the ship. These studies included screening of about half the population on Anuta, one of the most remote islands, and surveys of various villages and clinically ill individuals on other islands. Also carried out was the screening of almost all the students from many different Southern Solomon Islands and the examination of many patients at the government hospital on Santa Cruz. These studies will be useful in determining what patients should be evacuated for treatment of tuberculosis, as well as for aiding our understanding of the distribution of disease, such as tuberculosis and atypical mycobacterial infection throughout the islands. Correlation of chest x-ray findings with PPD and atypical mycobacterial and mycotic antigen skin sensitivities will be performed. In addition, some individuals were found to have roentgenographic evidence of cardiovascular disease. Two cases of probable initial stenosis, one case of pulmonary stenosis, and several people with cardiomegaly of undetermined origin were discovered. An incidental finding was the occurrence of calcium on the aortic arch in about the same frequency as occurs in the United States, suggesting that the absence of vascular disease syndromes in this population is not the result of an absence of atherosclerosis.

## VI. Parasitology: Malarial and Filarial and Intestinal Parasite Studies

Fifteen hundred thick and thin blood smears were taken on the island populations for evaluation of the incidence of parasitemia for malaria and filaria. Stool specimens in appropriate holding media provided a means for assessing the levels and kinds of helminthic and amoebic infectation in the populations. Hematological studies, including hemoglobin determinations and differential blood counts will also provide an index of anemia and of eosinophilia. Fluorescent antibody tests for malaria will be performed on selected population samples and correlated with the results of blood smear evaluations.

## Population Genetic Studies in Relation to Congenital Defects and Heredo-familial Diseases in the Isolated Island Populations

I. Blood group genetic factors and hemoglobinopathies.
Investigators: 1. D.C. Gajdusek, M.D.
2. R. Kirk, M.D.
3. M. Simons, M.D.
4. P.W. Brown, M.D.
5. R.A. Ferber, M.D.
6. S. Wiesenfeld, M.D.
7. R. Roos, M.D.
8. F. Cathala, M.D.
9. J. Sheridan, M.D.

II. Red cell enzyme pleomorphisms.
Investigators: 1. R. Kirk, M.D.
2. D.C. Gajdusek, M.D.
3. P.W. Brown, M.D.
4. R.A. Ferber, M.D.
5. F. Cathala, M.D.
6. R. Roos, M.D.
7. J. Sheridan, M.D.

III. Polymorphisms of serum protein factors.
Investigators: 1. R. Kirk, M.D.
2. D.C. Gajdusek, M.D.
3. P.W. Brown, M.D.
4. R.A. Ferber, M.D.
5. F. Cathala, M.D.
6. R. Roos, M.D.
7. J. Sheridan, M.D.

IV. Dermatoglyphics.
Investigators: 1. D. Rubinstein
2. C. Plato
3. D.C. Gajdusek, M.D.

V. Genetic distance studies between island groups.
Investigators: 1. S. Wiesenfeld, M.D.
2. R. Kirk, M.D.
3. R.A. Ferber, M.D.
4. D.C. Gajdusek, M.D.
5. J. Guiart, Ph.D.

A wide search for heredofamilial diseases and congenital abnormalities was carried out and genealogies collected on most of the island families. These data, taken together with laboratory data on pleomorphisms in erythrocyte surface antigens and red cell enzymes and serum proteins, the further genetic data on finger and palm prints, anthropometric measurements and color blindness, and from the medical survey for such genetic traits as albinism and pigmented rings around the optic disc, will all be used in studying the genetic structure of the populations from the different islands in relation to gene drift, and with respect to possible relationships, as indicated by their genetic similarities and dissimilarities. These data will be used to search for linkage between genetic constitution to the occurrence of various diseases or morphological traits.

## I. Blood Group Genetic Factors and Hemoglobinopathies

Clotted red cells are being analyzed for blood groups ABO, MNS, Rh, Kell, Duffy, Diego, Wright, and Lewis, by Dr. Malcolm Simons at the W.H.O. headquarters in Singapore, by Dr. Kenneth Brown and Mr. W.C. Leyshon at the National Institute of Dental Research, and by Dr. Robert Kirk at the Australian National University, Department of Human Biology. In addition, hemoglobin electrophoresis in search for hemoglobin A Tongariki, as well as other hemoglobin variants, is being carried out by Dr. Robert Kirk. These data, together with information on other polymorphic loci, will be used to determine genetic relationships and distinctions between the various island population studies. Data on the distribution of hemoglobin A Tongariki will be invaluable to the study of genetic drift and migration in the New Hebrides as well as data on other rare alleles among the red cell enzymes or other systems.

## II. Red Cell Enzyme Pleomorphisms

Red cell clots were obtained on over three thousand individuals from all the visited islands in the New Hebrides and the British Solomon Islands Protectorate. These samples are being analyzed in the Department of Human Biology, Australian National University, for polymorphisms at twenty different red cell enzyme loci. The resulting data will be used in conjunction with other genetic information we have gathered to analyze the degree of genetic isolation in these island populations. This data will also be used in our studies on genetic heterogeneity and gene drift, as well as in genetic distance summaries. They are potentially of great value in indicating or supporting suspected migration pattersn and will be used, together with other data, on pleomorphisms to calculate genetic distance and to search for linkage to various diseases.

## III. Polymorphisms of Serum Protein Factors

Over three thousand samples are being analyzed in the laboratory of Dr. Robert Kirk at the Australian National University for serum protein factors known to be polymorphic. These factors include transferrin, haptoglobin, albumin, group-specific protein (Gc), Gm and Inv allotypes of human immunoglobulin G (IgG). Part of each serum specimen will be maintained frozen at -70°C for possible future analysis of yet to be discovered polymorphisms. These data will further amplify the forces of genetic drift, migration and possible selective forces acting on these isolated island populations suspected of having as high a coefficient of kinship as can be found anywhere in the world.

### IV. Dermatoglyphics

Fifteen hundred subjects were palm- and finger-printed on both hands for dermatoglyphic analyses by Chris Plato at the National Institute of Dental Research. The data will be used to search for phenotypic differences in palm printing pattern and finger print pattern distributions and in ridge counting, in order to find evidence for relationships or genetic distinctions among the various island populations studied. An attempt is also being made to use dermatoglyphic data in genetic distance studies, employing erythrocyte antigens, serum protein and red cell enzyme pleomorphisms for the study of genetic heterogeneity and gene drift by genetic distance techniques.

### V. Genetic Distance Studies Between Island Groups

Information on close to thirty polymorphic loci, as well as dermatoglyphic phenotypes and kinship patterns, will be used in genetic distance analyses of the island populations surveyed. Genetic heterogeneity, as well as coefficients of kinship, will be determined. Graphic analyses, including the construction of genetic networks and principal components analysis, will also be attempted. Distinction between kinship, determined by bioassay (from polymorphism data) and that determined by migration patterns, dermatoglyphics and kinship patterns obtained from history, will be contrasted.

## Epidemiological Investigation of the Demography, Kinship, Migration, and Marriage Patterns of the Populations Studied; Documentation of the Ethnography, Natural History, Ecology, and Medical Procedures and Findings

I. Kinship systems and marriage and migration patterns.
Investigators: 1. J. Guiart, Ph.D.
2. D. Rubinstein
3. R. Feinberg
4. R. Lee, M.D.

II. Research cinema record.
Investigators: 1. D.C. Gajdusek, M.D.
2. J. Meyer
3. D. Rubinstein

III. Cataloguing and collating of still photography from the expedition.

Investigators: 1. J. Meyer
2. D. Rubinstein
3. I.G. Mbaginta'o
4. J. Kroeber
5. R.A. Ferber, M.D.
6. D.C. Gajdusek, M.D.

IV. Child drawings.

Investigators: 1. D. Rubinstein
2. J. Meyer
3. D.C. Gajdusek, M.D.

V. Recording of music.

Investigators: 1. D.C. Gajdusek, M.D.
2. D. Rubinstein
3. J. Kroeber

VI. Collection and documentation of ethnographic artifacts.

Investigators: 1. D. Rubinstein
2. J. Guiart, Ph.D.
3. J. Meyer
4. I.G. Mbaginta'o
5. D.C. Gajdusek, M.D.
6. P. Fetchko

## I. Kinship Systems and Marriage and Migration Patterns

Medical documentation for the entire three thousand some patients who were examined and bled included name, age, sex, village and island, also marital status and name of patient's spouse, names and villages and islands of the patient's parents, names of living children and reproductive histories of women, as well as travel and medical histories for each patient. Particular care was given to tracing the genealogical lines resulting from foreignera and immigrants to the islands during the last two or three generations. Individuals were questioned as to their assigned membership in exogemous, named social groupings (clans or lineages) and an attempt was made to elaborate the socially prescribed patterns of marriage among those groupings. This documentation makes possible a reconstruction of the genealogical and residential patterns on each island, and thus provides both an elucidation of the patterns of non-random mating and a measure of population dispersion among these isolated populations.

## II. Research Cinema Record

Cinema records from the expedition amount to more than eleven thousand feet of 16mm color film. Motion picture documentation was undertaken for several purposes: 1) cinema records were made of patients with neurological and other movement disorders to permanently record and permit further analysis of their conditions; 2) as a supplement to the medical survey and population genetic studies, cinema material will provide ethnographic background essential to interpreting the epidemiological and human biological observations and laboratory findings; 3) medical problems (other than neurological) which could be effectively recorded photographically were filmed, including elephantiasis, other filarial infection syndromes, albinism, congenital deformations, goiter and cretinism, and skin diseases; 4) as part of the Study of Child Growth and Development, cinema was used to record behavior patterns and early childhood learning; and 5) in accordance with the wishes of the Scripps Institution and the National Science Foundation, the field procedures of the expedition and the research use of the Alpha Helix facility in dealing with the island populations and handling of their medical problems were also filmed.

The entire eleven thousand foot corpus of motion picture film will be fully documented with supplementary material and will become Research Films of the Study of Child Growth and Development and Disease Patterns in Primitive Cultures of the National Institute of Neurological Diseases and Stroke in Bethesda.

## III. Cataloguing and Collation of Still Photography from the Expedition

Eleven of the expedition participants used one or more still cameras, documenting extensively every area of their work. There are more than eight thousand photographs, 90% of them in color. They fall into the following categories: 1) documentation of medical and genetic problems encountered, including filarial adenopathy, elephantiasis, tropical pyomyositis, tropical ulcers, trachoma, leprosy, gangosa yaws, goitrous cretinism, tinea versicolor and corporis, molluscum contagiosum, pedunculated warts, albinism and partial albinism, various congenital anomalies with and without mental retardation, club feet, reduction deformities, supernumerary digits, external ear deformities, diastasis recti, ptosis and atrophied extremities and post-poliomyelitis atrophy and paresis; 2) still photographic record of behavioral importance for the Study of Child Growth and Development, and to be used in longitudinal studies of child development; 3) identity photographs on almost every individual for whom we have a blood sample, for later correlation with human biological results, and for growth studies; 4) ethnographic background material to aid in the interpretation of genetic and medical findings, and for a general understanding of their cultural setting; and 5) photographs documenting the natural history and ecology of the islands for use in epidemiological studies and studies of disease ecology.

## IV. Child Drawings

Several hundred drawings were collected from children in most of the island groups studied. We have recorded the name, age and sex and village residence of each of the child artists. The child's depiction, frequently in a culturally stylized manner, of his island world and the activities within the village and on the sea, provide a useful and unique document for the study of child growth and development. These drawings are being used together with the large collection of drawings from different cultural and linguistic groups in Melanesia, especially New Guinea, Polynesia and Micronesia, which we have accumulated from children in low-acculturated societies over the past twenty years in the study of culturally determined symbolic styles and patterns of cognitive function.

### V. Recording of Music

Ten hours of island music, dancing and communal singing were recorded on magnetic tape and will be used largely as supplementary material to the corpus of cinema and flash still photography. On several islands we had the opportunity to make high-quality recordings of traditional Polynesian and Melanesian dance-songs and commemorative chants, which will become interesting ethnographic supplements to the studies of the human biology of these islands.

### VI. Collection and Documentation of Ethnographic Artifacts

Several hundred tools, household articles and ceremonial carvings were collected in the villages and district centers which the expedition visited. A principal aim of the photography during the expedition was to supplement this collection with a photographic record of several thousand artifacts in their village setting, particularly in reference to house architecture and settlement pattern, the use of indigenous tools, native art styles, tattoo patterns, canoe construction, traditional dress and the technology of gardening, irrigation and food preparation.

The collection and documentation of these artifacts will provide an ethnographic picture of the living conditions and dietary habits of the islanders, and will be important as background cultural data for studies of child rearing and disease patterns on the islands. The documentation of cultural styles of art and technology will also be useful as supplementary data to the medical studies of genetic distance among the island populations studied.

Publications:

Alpers, M.P. and Gajdusek, D.C. Problems of so-called "recoveries" and of differential diagnosis in kuru. N. Engl. J. Med., in press.

Alpers, M., Gajdusek, D.C., and Gibbs, C.J., Jr. Subacute spongiform encephalopathies, transmissible degenerative diseases of the nervous system. In Kakulas, B. (ed): Proceedings of the Second International Congress on Muscle Diseases. Perth, W. Australia, November 22-26, 1971. Excerpta Medica, Amsterdam, in press.

Alpers, M., Hunt, P., and Gajdusek, D.C. Genetic studies in relation to kuru. X. A genealogical study of Waisa village, of the South Fore, with an analysis of kuru incidence by generation. Am. J. Hum. Genet., in press.

Asher, D.M., Gajdusek, D.C., and Gibbs, C.J., Jr. Recent progress in the study of subacute spongiform encephalopathies. Vestn. Akad. Nauk SSSR, in press.

Asher, D.M., Gibbs, C.J., Jr., David, E., Alpers, M.P., and Gajdusek, D.C. Experimental kuru in the chimpanzee. Physical findings and clinical laboratory studies. In Proceedings of the Fourth International Congress of Primatology. Portland, Oregon, August 15-18, 1972. S. Karger AG, Basel, in press.

Asher, D., Gibbs, C.J., Jr., and Gajdusek, D.C. Focal chronic encephalitis: a critical review of the epilepsy partialis continua (Kozhevnikov's epilepsy) syndrome and related neurological disorders. Pediatrics, in press.

Asher, D.M., Gibbs, C.J., Jr., Lang, D.J., and Gajdusek, D.C. Persistent shedding of cytomegalovirus in the urine of healthy rhesus monkeys. Proc. Soc. Exp. Biol. Med., in press.

Beck, E., Daniel, M.P., Asher, D.M., Gajdusek, D.C., and Gibbs, C.J., Jr. Experimental kuru in the chimpanzee: a neuropathological study. Brain, in press.

Beck, E., Daniel, M.P., and Gajdusek, D.C. Human kuru: a pathological study of 40 brains. Brain, in press.

Benfante, R.J., Traub, R., Lim, K.A., Hooks, J., Gibbs, C.J., Jr., and Gajdusek, D.C. Serological reactions in kuru. Attempts to demonstrate immunological relationships between kuru virus and other known infectious agents. Am. J. Trop. Med. Hyg., in press.

Blake, N.M., Omoto, K., Kirk, R.L., and Gajdusek, D.C. Variation in red cell enzyme groups among populations of the Western Caroline Islands, Micronesia. Am. J. Hum. Genet., in press.

Blumberg, B., and Gajdusek, D.C. Genetic studies in relation to kuru. VIII. A study of two polymorphisms in the Eastern Highlands of New Guinea. Australia antigen in serum and β-amino butyric acid in urine. Am. J. Hum. Genet., in press.

Bobowick, A.R., Brody, J.A., Matthews, M.R., Roos, R., and Gajdusek, D.C. Creutzfeldt-Jakob disease: a case control study. Neurology, in press.

Brown, P., and Besancon, F. Effet du pH acide sur l'induction de l'état de résistance antivirale par l'intérferon. C.R. Acad. Sc. Paris, 275: 2275-2278, 1972.

Brown, P., and Besancon, F. Mécanisme d'inhibition à pH acide de l'induction de l'état de résistance antivirale par l'interferon. C.R. Acad. Sc. Paris, in press.

Brown, P., Besancon, F., and Chany, C. The effects of an acid pH upon the induction of antiviral resistance in vitro by interferon. Proc. Soc. Exp. Biol. Med. 142: 1195-1199, 1973.

Brown, P., and Cathala, F. Le kuru, modèle d'étude pour les maladies dégeneratives du systeme nerveux central. Encyclopedie Medico-Chirogicale, Tome du Systeme Nerveux, Premier Edition 3, p. 12f, 1972.

Brown, P., and Cathala, F. Le kuru: modèle epidémiologique pour l'étude des maladies dégénératives du système nerveux central. Med. Malad. Infec. (Paris) 2: 459-467, 1972.

Brown, P., Cathala, F., and Gajdusek, D.C. Further studies of viral antibodies in the cerebrospinal fluid of patients with multiple sclerosis: vaccinia and parainfluenza type 1. Proc. Soc. Exp. Biol. Med., in press.

Brown, P., and Chany, C. La résistance in vitro du visna à l'activité de l'interféron et l'absence de la production de l'interféron par le virus. C.R. Acad. Sci. Paris, in press.

Brown, P., Hooks, J., Roos, R., Gajdusek, D.C., and Gibbs, C.J., Jr. Attempt to identify the agent for Creutzfeldt-Jakob disease by antibody relationships to known viruses. Nature New Biol. 235: 149-152, 1972.

Brown, P., Sarragne, M., Chany, C., Gibbs, C.J., Jr., and Gajdusek, D.C. Tissue antagonist of interferon in normal and kuru or Creutzfeldt-Jakob infected primate brain. C.R. Acad. Sci. Paris, in press.

Brown, S.M., Gajdusek, D.C., Leyshon, W.C., Steinberg, A.G., Brown, K.S., and Curtain, C. Genetic studies in Paraguay: blood group, red cell, and serum genetic patterns of the Guayaki and Ayore Indians, Mennonite settlers and seven other Indian tribes of the Paraguayan Chaco. Am. J. Phys. Anthrop., in press.

Cathala, F., and Brown, P. La maladie de Creutzfeldt et Jakob. Transmission et etude virologique. Pathologie et Biologie, in press.

Cathala, F., and Brown, P. The possible virus aetiology of disseminated sclerosis. J. Clin. Pathol. (London), 25: 141-151, 1972.

Chany, C., Gregoire, A., Vignal, M., Lemaitre, J., Brown, P., Besancon, F., Suarez, H., and Cassingena, R. Mechanism of interferon uptake in parental and somatic monkey-mouse hybrid cells. Proc. Natl. Acad. Sci.USA 70: 557-561, 1973.

Chopra, H.C., Hooks, J.J., Walling, M.J., and Gibbs, C.J., Jr. Morphology of Simian foamy viruses with particular reference to virus isolated from spontaneous tumor of a rhesus monkey. J. Natl. Cancer Inst. 48: 451-463, 1972.

Chou, S.M., Roos, R., Burrell, R., Gutman, L., and Harley, J. Subacute and focal virus encephalitis. J. Neuropath. Exper. Neurol., in press.

Curtain, C.C., Gajdusek, D.C., O'Brien, D., and Garruto, R.M. Congenital defects of the central nervous system associated with hyperendemic goiter in a neolithic highland society of Western New Guinea. IV. Serum proteins and haptoglobins, transferrins, and hemoglobin types in goitrous and adjacent non-goitrous Western Dani. Hum. Biol. Oceania, in press.

Curtain, C.C., Gajdusek, D.C., Scheinberg, I.H., Alpers, M., and Hornabrook, R. Genetic studies in relation to kuru. IX. Serum protein and red cell polymorphisms in populations of the Eastern Highlands of New Guinea. Am. J. Hum. Genet., in press.

Ferber, R.A., Wiesenfeld, S.L., Roos, R., Bobowick, A.R., Gibbs, C.J., Jr., and Gajdusek, D.C. Familial Creutzfeldt-Jakob disease. Transmission of the familial disease to the chimpanzee. In Proceedings of the International Congress of Neurology. Barcelona, September 13-18, 1973. Excerpta Medica, Amsterdam, in press.

Ferber, R.A., Wiesenfeld, S.L., Roos, R., Bobowick, A.R., Gibbs, C.J., Jr., and Gajdusek, D.C. Transmission of familial form of Creutzfeldt-Jakob disease to laboratory primates, with a summary of kuru affected families. Neurology, in press.

Gajdusek, D.C. Spongiform virus encephalopathies. J. Clin. Pathol. (London) 25: 72-83, 1972.

Gajdusek, D.C. Colombian expedition to the Noanama Indians of the Rio Siguirisua and to the Cofan and Ingino tribes of the Putumayo. August 22, 1970 to September 14, 1970 (February 1972). National Institute of Neurological Diseases and Stroke.

Gajdusek, D.C. Melanesian Journal. Expeditions in West and East New Guinea, Solomon Islands, New Hebrides, Fiji and New Caledonia, to Study Child Growth and Development, Behavior and Disease Patterns, Human Genetics and Kuru. February 22, 1963 to July 23, 1963 (June 1972). National Institute of Neurological Diseases and Stroke.

Gajdusek, D.C. Slow virus infections and activation of latent virus infections in aging. Adv. Gerontol. Res. 4: 201-218, 1972.

Gajdusek, D.C. Kuru in the New Guinea Highlands. In Spillane, J.B. (ed.): Tropical Neurology, Chapter 29, 366-382, 1973. Oxford Press, London.

Gajdusek, D.C. Kuru and Creutzfeldt-Jakob disease: experimental models of non-inflammatory degenerative slow virus disease of the central nervous system. Ann. Clin. Res. (Helsinki), in press.

Gajdusek, D.C. Current status of research on human slow virus infections. Tenth Annual Meeting, Federation of Western Societies of Neurological Science, Colorado Springs, March 1-4, 1973. In-house Publication, V.A. Wadsworth General Hospital, Los Angeles, in press.

Gajdusek, D.C., and Alpers, M. Genetic studies in relation to kuru. I. Cultural, historical and demographic background. Am. J. Hum. Genet. 24: S1-S38, 1972.

Gajdusek, D.C., Alpers, M.P., and Gibbs, C.J., Jr. Kuru: epidemiological and virological studies of a unique New Guinean disease with wide significance to general medicine. In Hornabrook, R.W. (ed.): Symposium on Kuru. Institute of Human Biology, Goroka, Papua New Guinea, in press.

Gajdusek, D.C., Fetchko, P., Van Wyk, N.J., and Ono, S. Annotated Anga (Kukukuku) bibliography 1972. National Institute of Neurological Diseases and Stroke.

Gajdusek, D.C., and Gibbs, C.J., Jr. Transmissible virus dementias and kuru. The two subacute spongiform virus encephalopathies of man. Bull. Inst. Pasteur 70: 117-144, 1972.

Gajdusek, D.C., and Gibbs, C.J., Jr. Subacute and chronic diseases caused by atypical infections with unconventional viruses in aberrant hosts. In Pollard, M. (ed.): Perspectives in Virology 8 279-311, 1972. Academic Press, New York.

Gajdusek, D.C., and Gibbs, C.J., Jr. Discussions on scrapie, kuru and C-J disease: slow virus infections. In Sixty-fourth Ross Conference on Pediatric Research. Val-David, P.Q. Canada, June 1971. Ross Laboratories, Columbus, Ohio, pp. 156-222, 1972.

Gajdusek, D.C., and Gibbs, C.J., Jr. Transmission of kuru from man to the rhesus monkey (Macaca mulatta) 8 1/2 years following inoculation. Nature (London) 240: 5380, 1972.

Gajdusek, D.C., and Gibbs, C.J., Jr. Transmissible virus dementias. In de la Fuente, R., and Weisman, M.N. (eds.): Proceedings, V World Congress of Psychiatry. Mexico D.F., November 28-December 4, 1971. Excerpta Medica, Amsterdam, in press.

Gajdusek, D.C., and Gibbs, C.J., Jr. Kuru and the virus dementias. In Biohazards in Cancer Research. Cold Spring Harbor Press, New York, in press.

Gajdusek, D.C., Gibbs, C.J., Jr., Rogers, N.G., Basnight, M., and Hooks, J. Persistence of viruses of kuru and Creutzfeldt-Jakob disease in tissue cultures of brain cells. Nature (London) 235: 104-105, 1972.

Gajdusek, D.C., and Zigas, V. Clinical laboratory studies of kuru patients. Papua New Guinea Medical Journal, in press.

Gajdusek, D.C., and Garruto, R.M. Congenital defects of the central nervous system associated with hyperendemic goiter in a neolithic highland society of Western New Guinea. III. Total iodine and protein bound iodine levels in goitrous and adjacent non-goitrous populations. Human Biol. Oceania, in press.

Garruto, R.M., and Gajdusek, D.C. The West Irian focus of hyperendemic goitrous cretinism and associated deaf mutism. In Proceedings of the IX International Congress of Anthropology and Ethnology, August 1973, Chicago, in press.

Gavrilov, V.I., Asher, D.M., Vyalushkina, S.D., Ratushkina, L.S., Zmieva, R.G., and Tumyan, B.G. Persistent infection of a continuous line of pig kidney cells with a variant of the WSN strain of influenza A virus. Proc. Soc. Exper. Biol. Med. 140: 109-117, 1972.

Gibbs, C.J., Jr., and Gajdusek, D.C. Transmission of scrapie to the cynamolgus monkey (Macaca fascicularis). Nature 236: 73-74, 1972.

Gibbs, C.J., Jr., and Gajdusek, D.C. Amyotrophic lateral sclerosis, Parkinson's disease and the amyotrophic lateral sclerosis-Parkinsonism-dementia complex on Guam: a review and summary on attempts to demonstrate infection as the etiology. J. Clin. Pathol. (London) 25: 132-140, 1972.

Gibbs, C.J., and Gajdusek, D.C. Isolation and characterization of the subacute spongiform virus encephalopathies of man: kuru and Creutzfeldt-Jakob disease. J. Clin. Pathol. (London) 25: 84-96, 1972.

Gibbs, C.J., Jr., and Gajdusek, D.C. Neurologic diseases of man with slow virus etiology. In Day, S.B. and Good, R.A. (eds.): Membranes and Viruses in Immunopathology, pp. 397-409, 1972. Academic Press, New York.

Gibbs, C.J., Jr., and Gajdusek, D.C. Experimental subacute spongiform virus encephalopathies in primates. Science, in press.

Gibbs, C.J., Jr., and Gajdusek, D.C. Biology of kuru and Creutzfeldt-Jakob disease. In Proceedings of the A.A.P.B. Symposium on Slow Virus Infections, Washington, D.C., February 26-27, 1973. Williams and Wilkins, Baltimore, in press.

Gibbs, C.J., Jr., and Gajdusek, D.C. Cell-virus interactions in slow infections of the nervous system. In Neurosciences: Third Study Program, Neurosciences Research Program, Boulder, Colorado, July 24-August 11, 1972. M.I.T. Press, Brookline, Mass., in press.

Gibbs, C.J., Jr., and Gajdusek, D.C. Scrapie virus. Virology, in press.

Hooks, J., Gibbs, C.J., Jr., et al. Relationship of the Griswald oncornavirus to the Mason and Pfizer viruses. Virology, in press.

Hooks, J., Gibbs, C.J., Jr., Chopra, H., Lewis, M., and Gajdusek, D.C. Spontaneous transformation of human brain cells grown in vitro and characterization of associated virus particles. Science 176: 1420-1422, 1972.

Hooks, J., Gibbs, C.J., Jr., Chou, S., Howk, R., Lewis, M., and Gajdusek, D.C. Isolation of a new Simian foamy virus from a spider monkey brain culture. J. Infec. Immun., in press.

Hooks, J., Gibbs, C.J., Jr., Chou, S., Lewis, M., and Gajdusek, D.C. Isolation of a new Simian foamy virus from a spider monkey brain culture. In Bacteriological Proceedings, Seventy-third Annual Meeting of the American Society for Microbiology, Miami, May 6-11, 1973, in press.

Hooks, J., Gibbs, C.J., Jr., Cutchins, E.C., Rogers, N.G., Lampert, P., and Gajdusek, D.C. Characterization and distribution of two new foamy viruses isolated from chimpanzees. Arch. Gesamte Virusforsch. 38: 38-55, 1972.

Hooks, J., Gibbs, C.J., Jr., and Gajdusek, D.C. Reply to Katz, M., Koprowski, H., and Moorhead, P.S. Transformation of cell cultures derived from human brain. Science 179: 1019-1020, 1973.

Kirk, R.L., McDermid, E.M., Blake, N.M., Gajdusek, D.C., Leyshon, W.C., and MacLennan, R. Serum protein and red cell enzyme groups of three Amerindian populations in Colombia. Am. J. Hum. Genet., in press.

Kitchin, F.D., Bearn, A.G., Alpers, M., and Gajdusek, D.C. Genetic studies in relation to kuru. III. The distribution of the inherited serum group specific protein (Gc) phenotypes in New Guineans: an association of kuru and GcAb phenotypes. Am. J. Hum. Genet. 24: S72-S85, 1972.

Lampert, P.W., Gajdusek, D.C., and Gibbs, C.J., Jr. Subacute spongiform virus encephalopathies. Scrapie, kuru and Creutzfeldt-Jakob disease. Am. J. Pathol. 68: 626-646, 1972.

Lewis, Monica, Frye, L., Gibbs, C.J., et al. Herpes viruses from capuchin monkeys. Proc. Soc. Exper. Biol. Med., in press.

Mbaginta'o, I. Funeral ceremonies in the Anga Dunkwi region. J. Soc. des Oceanistes, in press.

Mbaginta'o, I. Medicine spirits of the Dunkwi Anga. J. Soc. des Oceanistes, in press.

Niedermeyer, E., Gibbs, C.J., Jr., Marsh, R., and Gajdusek, D.C. EEG studies in subacute and degenerative neurological diseases experimentally produced in ferrets and chimpanzees. Electroencephalogr. Clin. Neurophysiol. 33: 351-352, 1972.

Peterson, D.A., Wolfe, L.G., Deinhardt, F., Gajdusek, D.C., and Gibbs, C.J., Jr. Transmission of kuru and Creutzfeldt-Jakob disease to marmoset monkeys. In Bacteriological Proceedings, Seventy-third Annual Meeting of the American Society for Microbiology, Miami, May 6-11, 1973, in press.

Plato, C.C., Brown, P., and Gajdusek, D.C. Dermatoglyphics of the Micronesians from the Outer Islands of Yap. Z. Morphol. Anthropol. 64: 29-44, 1972.

Plato, C.C., and Gajdusek, D.C. Dermatoglyphics of the Asmat and Tjitak peoples of West New Guinea: with a note on the R/U ratio of the C line of the palm. Hum. Biol. Oceania 1: 255-258, 1972.

Plato, C.C., and Gajdusek, D.C. Genetic studies in relation to kuru. IV. Dermatoglyphics of the Fore and Anga populations of the Eastern Highlands of New Guinea. Am. J. Hum. Genet. 24: S86-S94, 1972.

Roos, R., Chou, S.M., Rogers, N.G., Basnight, M., and Gajdusek, D.C. Isolation of an adenovirus 32 strain from human brain in a case of subacute encephalitis. Proc. Soc. Exper. Biol. Med. 139: 636-640, 1972.

Roos, R., Gajdusek, D.C., and Gibbs, C.J., Jr. The clinical characteristics of transmissible Creutzfeldt-Jakob disease. Brain 96: 1-20, 1973.

Simmons, R.T., Graydon, J.J., Gajdusek, D.C., Alpers, M.P., and Hornabrook, R.W. Genetic studies in relation to kuru. II. Blood group genetic patterns in kuru patients and populations of the Eastern Highlands of New Guinea. Am. J. Hum. Genet. 24: S39-S71, 1972.

Simmons, R.T., Graydon, J.J., Rodrigue, R.B., and Gajdusek, D.C. Blood group genetic data from the Western Highlands District, New Guinea, the adjacent Southern Highlands District and Western District, Papua. Am. J. Phys. Anthrop., in press.

Steinberg, A.G., Gajdusek, D.C., and Alpers, M. Genetic studies in relation to kuru. V. The distribution of human gamma globulin allotypes in New Guinea populations. Am. J. Hum. Genet. 24: S95-S110, 1972.

Wallace, G.D., Zigas, V., and Gajdusek, D.C. Toxoplasmosis and cats in New Guinea. Am. J. Trop. Med. Hyg., in press.

Wiesenfeld, S.L., and Gajdusek, D.C. Genetic studies in relation to kuru. VI. Genetic structure and heterozygosity in the Eastern Highlands of New Guinea. Am. J. Hum. Genet., in press.

Wiesenfeld, S.L., Ferber, R.A., Harpending, H.C., and Gajdusek, D.C. Genetic analysis of a slow virus infection. Genetic differnetiation among kuru and normal Fore. In Proceedings of the IXth International Congress of Anthropological and Ethnological Sciences, Chicago, August 28-September 8, 1973, in press.

Yu, R.K., Ledeen, R.W., Gajdusek, D.C., and Gibbs, C.J., Jr. Ganglioside changes in slow virus diseases: analysis of chimpanzee brains infected with kuru and Creutzfeldt-Jakob agents. Brain Res., in press.

Zigas, V., and Benfante, R.J. Human toxoplasmosis: an evaluation of current progress. Trop. Geogr. Med. 24: 1-6, 1972.

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